BD FACSArray System User's Guide

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History

Revision	Date	Change Made
335634 Rev. A	11/03	Initial release

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About This Guide

This guide describes how to set up and operate the BDFACSArray™ bioanalyzer and BD FACSArray system software.

The compact BD FACSArray system provides plate-based analysis of proteins in both cellular and BD™ Cytometric Bead Array (CBA) assays.

The *BD FACSArray System User's Guide* assumes you have a working knowledge of basic Microsoft® Windows® operation. If you are not familiar with the Windows operating system, refer to the documentation provided with your computer.

Before using BD FACSArray system software, review the ReadMe file by double-clicking the BD FACSArray ReadMe icon on the desktop. This file contains important information that is not printed in this user's guide.

Conventions

The following tables list conventions used throughout this guide. Table 1 lists the symbols that are used to alert you to a potential hazard. The word accompanying the hazard symbol indicates the level of severity, as shown in Table 2. Text and keyboard conventions are shown in Table 3.

Table 1 Hazard icons

Symbol	Hazard	Symbol	Hazard
	Electrical		Biological risk
	Laser radiation		General

Table 2 Severity indicators[a]

Indicator	Level of Severity
WARNING	The hazard or unsafe practice could result in severe injury or death.
CAUTION	The hazard or unsafe practice could result in minor injury.
NOTICE	The hazard or unsafe practice could result in a possibly dangerous situation; material could be damaged or data could be lost.

a. In compliance with the ANSI Z535.4 standard: Product Safety Signs and Labels

Table 3 Text and keyboard conventions

Convention	Use
☑ **Tip**	Highlights features or hints that can save time and prevent difficulties
Italics	Italics are used to highlight book titles and new or unfamiliar terms on their first appearance in the text.
>	The arrow indicates a menu choice. For example, "choose File **>** Print" means to choose Print from the File menu.
Command-*X*	When used with key names, a dash means to press two keys simultaneously. For example, Command-P means to hold down the Command key while pressing the letter *p*.

Technical Assistance

For technical questions or assistance in solving a problem:

- Read the section of the user's guide specific to the operation you are performing.
- See Chapter 10, Troubleshooting.

If additional assistance is required, contact your local BD Biosciences technical support representative or supplier.

When contacting BD Biosciences, have the following information available:

- product name and serial number

  Open the front compartment door; information is located on the panel above the sheath and waste tasks.
- any error messages
- details of recent system performance

For instrument support from within the US, call (877) 232-8995, prompt 2, 2.

For support from within Canada, call (888) 259-0187.

Customers outside the US and Canada, contact your local BD representative or distributor.

Safety and Limitations

The BD FACSArray bioanalyzer is equipped with safety features for your protection. Operate the instrument only as directed in the user's guide. Do not perform instrument maintenance or service except as specifically stated. Keep this safety information available for reference.

Laser Safety

WARNING Use of controls, adjustments, or procedures other than those specified in the user's guide might result in hazardous radiation exposure.

Lasers or laser systems emit intense, coherent electromagnetic radiation that has the potential of causing irreparable damage to human skin and eyes. The main hazard of laser radiation is direct or indirect exposure of the eye to thermal radiation from the visible and near-infrared spectral regions (325–1,400 nm). Direct eye contact can cause corneal burns, retinal burns, or both, and possible blindness.

Laser Classification

Laser hazard levels depend on laser energy content and the wavelengths used. A numbered system is used to categorize lasers according to different hazard levels. The higher the classification number, the greater the potential hazard. The BD FACSArray bioanalyzer is a Class 1 (I) laser product per 21 CFR Subchapter J and EN 60825-1: 1994 + A1: 2003 + A2: 2001: IEC 60825-1: 1993/A2: 2001. The lasers are fully contained within the instrument structure and call for no special work area safety requirements except during service procedures. These procedures are to be carried out only by BD Biosciences service personnel.

Precautions for Safe Operation

WARNING Modification or removal of the outer cover of the instrument or the laser shields could result in exposure to hazardous laser radiation. To prevent irreparable damage to human skin and eyes, do not remove the outer cover of the instrument or the laser shields or attempt to service the instrument. See Precaution Labels on page xviii.

WARNING Laser power up to 15 mW at 532 nm and up to 15 mW at 635 nm could be accessible in the interior of the bioanalyzer.

Electrical Safety

⚠ **WARNING** Many portions of the electrical system, including the printed circuit boards, are at a dangerous voltage level. To prevent shock injury or damage to the instrument, follow these guidelines.

- Connect the equipment to an approved power source only. Do not use extension cords. If cords, plugs, or cables are damaged, immediately contact BD Biosciences for troubleshooting or a replacement.
- Do not remove the grounding prong from the power plug. Have a qualified electrician replace any ungrounded receptacles with properly grounded receptacles in accordance with the local electrical code.
- **Turn off the power switch on the back of the instrument and unplug the power cord** before servicing or performing maintenance on the instrument, unless otherwise noted.
- For installation outside the US, use the proper power cord.
- If you detect fluid leaking from the BD FACSArray bioanalyzer or cytometer, immediately turn off the instrument and contact BD Biosciences for assistance.

⚠ **WARNING** Protect against the risk of fire by replacing fuses only with those of the specified type and rating.

Biological Safety

☣ **WARNING** All biological specimens and materials coming into contact with them can transmit potentially fatal disease. To prevent exposure to biohazardous agents, follow these guidelines.

- Handle all biological specimens and materials as if capable of transmitting infection. Dispose of waste using proper precautions and in accordance with local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

- Expose waste container contents to bleach (10% of total volume) before disposal. Dispose of waste in accordance with local regulations. Use proper precautions and wear suitable protective clothing, eyewear, and gloves.

- Prevent waste overflow by emptying the waste container daily or whenever prompted by BD FACSArray software.

- If you detect fluid leaking from the BD FACSArray bioanalyzer, immediately turn off the instrument and contact BD Biosciences for assistance.

For information on laboratory safety, refer to the following guidelines. NCCLS documents can be ordered online at www.nccls.org.

- *Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue; Approved Guideline*. Wayne, PA: National Committee for Clinical Laboratory Standards, 1997. NCCLS document M29-A.

- *Procedures for the Handling and Processing of Blood Specimens; Approved Guideline*. Wayne, PA: National Committee for Clinical Laboratory Standards; 1990. NCCLS document H18-A.

General Safety

⚠ **WARNING** The BD FACSArray bioanalyzer is equipped with a safety feature that prevents operation of the instrument when the plate door is open. Do not attempt to override this safety feature. Keep the plate door closed during processing to prevent injury from moving parts or potentially biohazardous materials.

⚠ **WARNING** To prevent personal injury or damage to the instrument, do not attempt to move the BD FACSArray bioanalyzer. Contact BD Biosciences for assistance.

⚠ **CAUTION** Movement of mechanical parts within the instrument can pinch or injure your hands or fingers. To prevent injury by moving parts, follow these precautions.

- Keep your hands away from the plate holder once you position the plate on it. The plate holder will automatically retract when you click Load.
- Keep your hands away from the plate door during instrument operation.

⚠ **NOTICE** Attempting to use multiwell plates other than those specified in the BD FACSArray Plate Definition Guide (packaged with your user's guide) can damage the instrument.

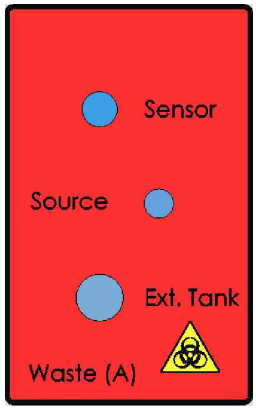
⚠ **NOTICE** Keep all instrument doors closed during operation to avoid potential electrical interference.

Limitations

- For Research Use Only. Not for use in diagnostic or therapeutic procedures.
- For sample and reagent limitations, refer to the appropriate reagent package insert.
- Not all multiwell plates have been qualified for use on the BD FACSArray bioanalyzer. For a list of compatible multiwell plates, refer to the BD FACSArray Plate Definition Guide.
- The BD FACSArray instrument was not designed to support deep-well plate analysis.

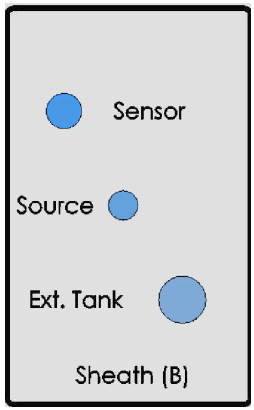
Precaution Labels

The following precaution labels appear on the BD FACSArray bioanalyzer to indicate a potential hazard. Do not remove these labels. Use appropriate precautions to avoid injury by the indicated hazard. See the previous sections in this guide for more information.

Label	Location(s)	Potential Hazard
	Waste (A) tank, waste (A) tank compartment, and I/O panel	Risk of exposure to biologically transmissible disease
	Waste (A) tank cap	Risk of exposure to biologically transmissible disease
	Back wall of waste (A) tank compartment	Risk of exposure to biologically transmissible disease Sensor—waste (A) sensor probe connector Source—waste (A) tubing connector Ext—external waste (A) tank connector

Label	Location(s)	Potential Hazard
 CAUTION VISIBLE AND/OR INVISIBLE CLASS 3B LASER RADIATION WHEN OPEN. AVOID EXPOSURE TO THE BEAM. 335749	On or near all front and side laser shields and anywhere the laser beam can emerge from the instrument	Risk of exposure to hazardous laser radiation
 CAUTION: MOVING PARTS 334584	On or near the shutter assembly	Risk of crushing or pinching by moving parts

Information Labels

Label	Location(s)	Meaning
	Sheath (B) tank, sheath (B) tank compartment, and I/O panel	Sheath fluid
	Sheath (B) tank cap	Sheath fluid
	Back wall of sheath (B) tank compartment	Sensor—sheath (B) sensor probe connector Source—sheath (B) tubing connector Ext—external sheath (B) tank connector

1

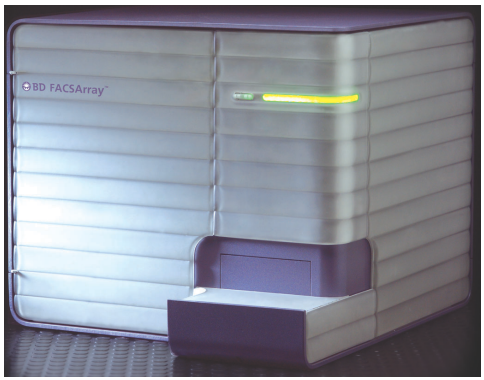
Introduction

The following topics are covered in this chapter:

- System Overview on page 22
- System Requirements on page 23
- Compatibility on page 24
- What's Installed on page 25
- System Administration on page 25
- Reinstalling the Software on page 30

System Overview

The BD FACSArray system consists of the BD FACSArray bioanalyzer, computer workstation, BD FACSArray system software, and BD Cytometric Bead Array (CBA) software. The system was developed for fast, high-content analysis of proteins in both cellular- and bead-based assays using 96-well plates. Instrument setup and acquisition are simplified because all instrument controls are within the software.



System highlights:

- New plate sampling technology provides efficient sample acquisition.
- Digital electronics process up to 15,000 events per second.
- The optical detection unit measures four fluorescent colors, plus forward scatter (FSC) and side scatter (SSC), providing multi-parameter sample analysis.
- Default instrument settings for selected applications decrease sample setup time.
- Software tracks user identity with log in and log out times, facilitating security and traceability.

System Requirements

The following sections describe hardware and software requirements for running the software.

Hardware

- BD FACSArray bioanalyzer
- PC workstation purchased through BD Biosciences

  Workstation will be equipped with a minimum 2.4 GHz Pentium® 4 processor with a least 1 GB of RAM, 60 GB of available hard-drive space, the Microsoft Windows 2000 Professional (US-English) operating system, and a 17-inch flat screen monitor.

⚠ **NOTICE** This version of BD FACSArray system software has been validated for use on the BD FACSArray workstation running Microsoft Windows 2000 Professional (US-English) operating system. Regional options allowing comma usage by the operating system can corrupt the user login tracking document and optical spillover matrix. Contact your local BD Biosciences representative before making any alterations to the system.

⚠ **NOTICE** Workstation requirements are subject to change. Contact your BD Biosciences sales representative for up-to-date requirements.

Software

The following software is automatically installed by and is required to run BD FACSArray system software.

- Java™ 2 Runtime Environment
- Sybase™ SQL Anywhere™ Studio
- Adobe® Acrobat® Reader 6

Compatibility

See the following sections for data file and other software compatibility.

Data Files

BD FACSArray software can export FCS 2.0 or 3.0 data files that can be analyzed by other software applications such as BD CBA, BD CellQuest Pro, BD FACSDiva, and FlowJo™ software.

⚠ **NOTICE** Use BD FACS™ Convert software to translate certain exported FCS 2.0 files into a Macintosh®-compatible format. BD CellQuest Pro software can analyze FCS 2.0 data files.

⚠ **NOTICE** BD FACSArray system software cannot open BD CellQuest Pro Experiment documents or import BD FACSDiva experiments.

⚠ **NOTICE** Only experiments created with BD FACSArray system software can be imported. FCS files cannot be imported into BD FACSArray system software.

What's Installed

The software installer places the following components on the computer hard drive. The installer for each application is launched automatically during software installation. If any of these components is already installed, the installer skips to the next installation step.

- BD FACSArray System software, for acquiring and analyzing data
- Java2 Runtime Environment (JRE), for running BD FACSArray system software
- BD FACSArray Data Management Utility, for backing up and restoring data
- Sybase SQL Anywhere Studio, needed to run the database
- Adobe Acrobat Reader, needed to view the PDF version of the user's guide
- BD Cytometric Bead Array (BD CBA) software version 1.4 or greater
- Microsoft Excel software version XP or greater with Solver Add-In
- Windows Explorer

System Administration

Windows 2000 Software

⚠ **NOTICE** Your BD FACSArray computer has been configured with a Microsoft Windows account with administrative privileges. The account password will be given to the lab director by the field service engineer during installation of the bioanalyzer.

To install BD FACSArray system software updates, add and delete users, modify attributes of a user's password or status, and grant administrative privileges to another user, you must have Windows administrative access to the software.

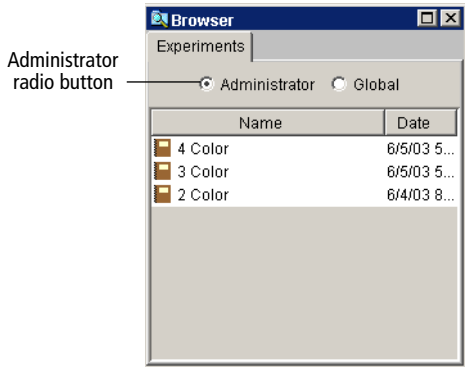
BD FACSArray System Software

BD FACSArray system software recognizes three user types.

- Administrator—can view and use all experiments
- User with Administrator privileges—can view and use all experiments
- User without Administrator privileges—can view and use only user-owned and shared experiments

The BD field service engineer will set up an Administrator for the software during installation of the BD FACSArray bioanalyzer. It is up to your lab director to decide who should have administrative access to the software.

Clicking the Administrator button in the Browser allows you to see all experiments, even those marked private.



Adding New Users

In order to add new users, you must have administrative access to the software.

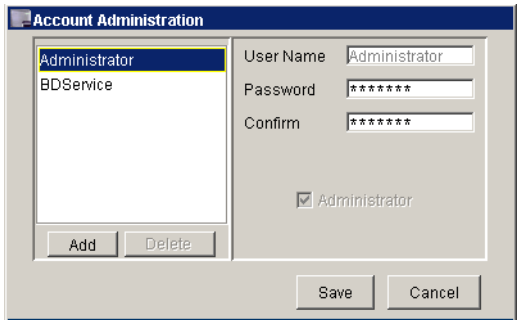
1 Start the software.

See Software Startup on page 40.

2 At the User Login dialog, enter the Administrator name and password.

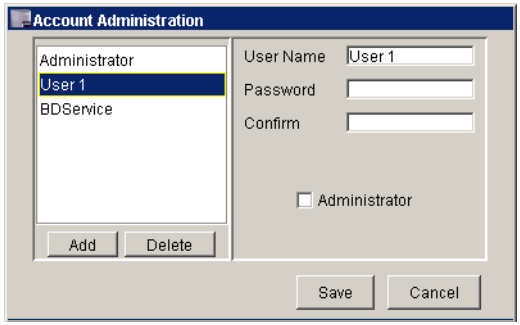
3 Choose File > Administration.

The following dialog appears.



4 Click the Add button.

User 1 appears in the User Name field.



5 Click inside the User Name field to select the text, and then enter a new user name.

6 Enter the new password in the Password field.

You can have a blank password.

7 Enter the new password in the Confirm field.

8 If you want the new user to have Administrator privileges, click the Administrator checkbox to select it.

Assigning Administrator privileges allows the user to see and delete all experiments.

9 Click the Save button.

Deleting Users

Before deleting a user name, delete all experiments belonging to the user.

1 If BD FACSArray system software is open, quit the program.

2 Restart the software.

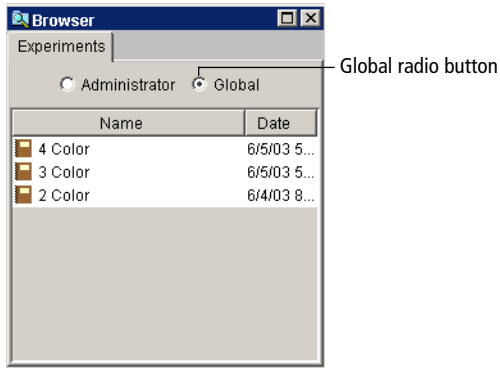
See Software Startup on page 40.

3 At the User Login dialog, enter the name and password of the user you want to delete.

4 In the Browser, click the Global radio button (Figure 1-1 on page 29).

Clicking Global restricts the Browser to displaying experiments that only the user owns or shares.

Figure 1-1 Browser showing Global radio button selected

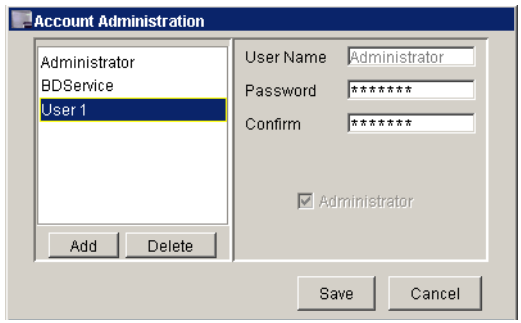


5 Delete all experiments owned by the user.

Follow instructions for deleting experiments on page 281.

6 Choose File > Administration.

7 From the User list, select the name you want to delete.



8 Click Delete.

The name is deleted from the list.

If you decide not to delete the name, click Cancel.

9 Click Save to exit the dialog and save the changes.

Reinstalling the Software

⚠ **NOTICE** To prevent the loss of instrument settings established during manufacturing, **do not** install a new database during software installation on an existing acquisition workstation; choose to use the existing database. Contact BD Biosciences for assistance.

BD FACSArray system software has already been installed on your computer workstation. If you need to reinstall the software on your acquisition workstation (the computer attached to your BD FACSArray bioanalyzer), do not install a new database during software installation. See step 13 on page 34.

⚠ **NOTICE** For up-to-date installation information, review the BD FACSArray ReadMe file.

⚠ **NOTICE** This version of BD FACSArray system software has been validated for use on the BD FACSArray workstation running Microsoft Windows 2000 Professional (US-English) operating system. Regional options allowing comma usage by the operating system can corrupt the user login tracking document and optical spillover matrix. Please contact your local BD Biosciences representative before making any alterations to the system.

⚠ **NOTICE** Make sure no programs are running that might conflict with the installer software.

Choosing Installation Options

1 If you are reinstalling the software on an existing workstation, make a backup copy of your current database; if you are installing the software on a new analysis workstation, proceed to step 2.

⚠ **NOTICE** To prevent data loss, make a backup copy of your current database before you reinstall the software on an existing workstation.

See Chapter 8, Data Management, on page 275, for instructions on backing up data.

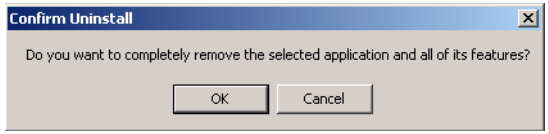
2 Insert the BD FACSArray System Software installation CD into the CD-ROM drive.

If the installer does not start up automatically, use Windows Explorer to view the CD contents, find, and double-click the Setup.exe icon.

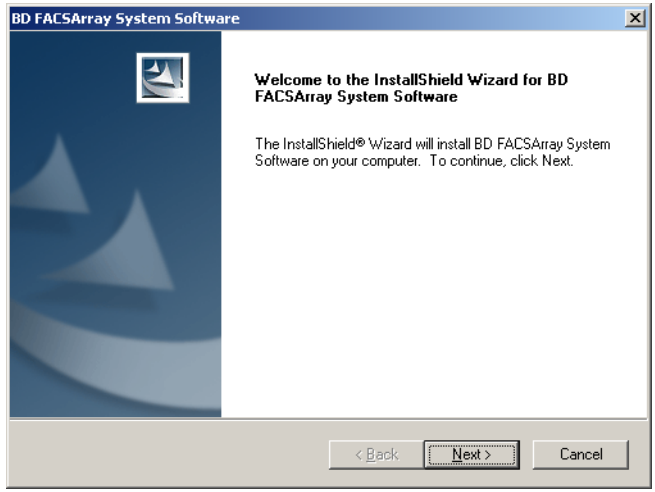
3 If you are attempting to install the software on a workstation that uses a non-US–English operating system, the following message appears.

BD FACSArray System Software
Application can be installed only on US English language platform. Please contact Technical Support.
OK

4 If you are reinstalling the software on an existing workstation, click OK when the following message appears.



5 When the BD FACSArray System Software Welcome screen appears, click Next.


BD FACSArray System Software
Welcome to the InstallShield Wizard for BD FACSArray System Software
The InstallShield® Wizard will install BD FACSArray System Software on your computer. To continue, click Next.
< Back
Next >
Cancel

6 After reading the licensing information, click Yes to accept the license agreement and continue installation.

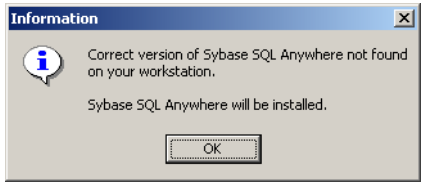
7 Review the installation instructions; click Next.

The installer checks to see if the correct version of Java Runtime Environment is installed on the hard drive; if not, the following dialog appears.

Information
Correct version of Java Runtime Environment not found on your workstation.
Java Runtime Environment will be installed.
OK

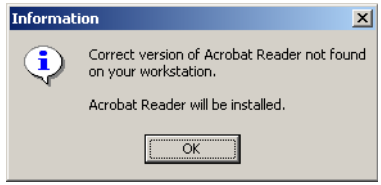
8 Click OK to install Java Runtime Environment.

The installer checks to see if the correct version of Sybase SQL Anywhere is installed on the hard drive; if not, the following dialog appears.


Information
Correct version of Sybase SQL Anywhere not found on your workstation.
Sybase SQL Anywhere will be installed.
OK

9 Click OK to install Sybase SQL Anywhere.

If the correct version of Adobe Acrobat Reader is not installed on the hard drive, the following dialog appears.

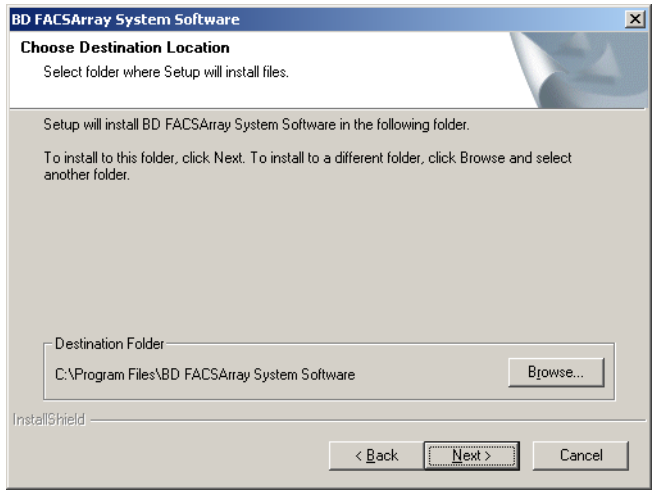


10 Click OK to install Adobe Acrobat Reader.

Adobe Acrobat Reader is needed to view the PDF version of the user's guide.

11 Verify the destination folder for BD FACSArray system software; click Next.

⚠ **NOTICE** When installing new software, ensure that the destination folder does not reside on a removable media drive such as a Zip, CDW, or DVD drive. If you need to change the drive, keep the same folder names and paths.


BD FACSArray System Software
Choose Destination Location
Select folder where Setup will install files.
Setup will install BD FACSArray System Software in the following folder.
To install to this folder, click Next. To install to a different folder, click Browse and select another folder.
Destination Folder
C:\Program Files\BD FACSArray System Software
Browse...
InstallShield
< Back
Next >
Cancel

By default, the application is installed in the Program Files\BD FACSArray System Software folder on the C: drive.

12 Verify the destination folder for the database; click Next.

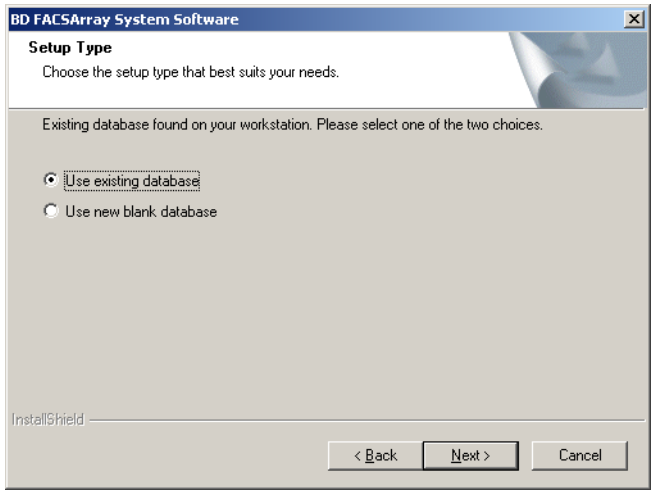
By default, the database is installed in the BDDatabase\BD FACSArray System Software folder on the D: drive.

13 If the following software dialog appears (Figure 1-2), choose to use the existing database; click Next.

⚠ **NOTICE** To prevent the loss of instrument settings established during manufacturing, **do not** install a new database during software installation on an existing acquisition workstation. Contact BD Biosciences for assistance.

This software dialog appears only if you are reinstalling the software on an existing workstation.

Figure 1-2 Database choice screen


BD FACSArray System Software
Setup Type
Choose the setup type that best suits your needs.
Existing database found on your workstation. Please select one of the two choices.
Use existing database
Use new blank database
InstallShield
< Back
Next >
Cancel

14 Click Next to view a summary of the installation options.

To alter installation options, click Back until you reach the appropriate screen, change the option, and then click Next until you reach the summary screen.

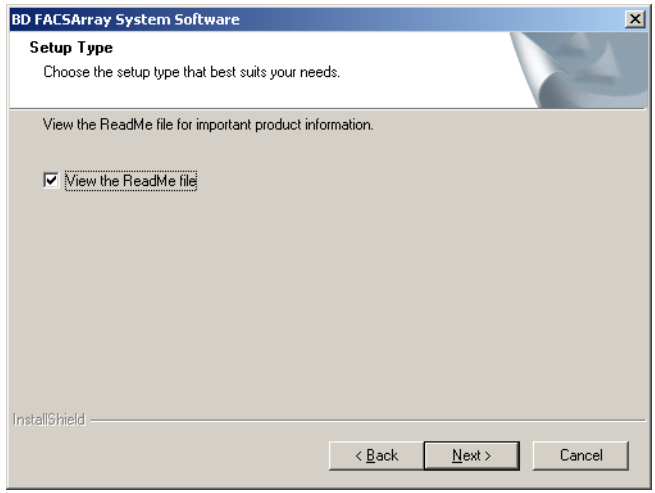
Copying Software Files

1 Click Next to begin copying the files.

The installer loads BD FACSArray System software and its support files on the specified drives.

When all required files have been installed, the following dialog appears.

Figure 1-3 ReadMe file access screen


BD FACSArray System Software
Setup Type
Choose the setup type that best suits your needs.
View the ReadMe file for important product information.
View the ReadMe file
InstallShield
< Back
Next >
Cancel

2 Click Next to review the ReadMe file.

The ReadMe file contains important software information that is not in this user's guide. Review the file and then click the close box to return to Software Setup.

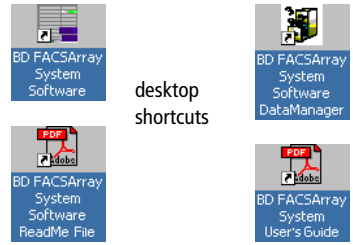
3 Click Finish to complete the installation.

BD Biosciences recommends that you reboot the computer before starting the application for the first time.

⚠ **NOTICE** If you are upgrading from a previous version of the software, restart the instrument and computer before launching the software.

Reviewing Files Installed

The installer places shortcuts to BD FACSArray System Software, BD FACSArray Data Manager, BD FACSArray System ReadMe file, and the BD FACSArray System User's Guide on the desktop. These shortcuts are also added to the Start menu (Start > Programs > BD FACSArray System Software...).


BD FACSArray System Software
BD FACSArray System Software ReadMe File
desktop shortcuts
BD FACSArray System Software DataManager
BD FACSArray System User's Guide

BD FACSArray system software and most supporting files are placed in the Program Files folder on the specified drive. You will find a copy of the ReadMe file and supporting documentation, including a PDF file of this user's guide in the Program Files\BD FACSArray System Software folder. You will also find a Templates folder that contains predefined BD templates. Any templates you create will be saved to this folder. See Choosing a Wizard Session on page 204 for instructions on accessing the templates.

Table 1-1 Useful files located in the BD FACSArray System Software folder

File	Folder	Function
BD FACSArray System Software.exe	BD FACSArray System Software	BD FACSArray system software executable
DataManager.exe	BD FACSArray System Software	Data Management Utility executable
Wizard sessions	Wizard	Wizard templates
audit_log.txt	Log	User login tracking information
Console.log	Log	Event_LogStyle.css for BD Biosciences troubleshooting purposes
ReadMe.doc	BD FACSArray System Software	Information not included in user's guide
User's Guide.pdf	BD FACSArray System Software	PDF file of user's guide

⚠ **NOTICE** If you move DataManager.exe out of the BD FACSArray system software application directory or run it from a batch file outside the BD FACSArray system software directory, it might not work correctly. DataManager references directories using the BD FACSArray system software application directory as home base.

The following files are placed in the BDDatabase\BD FACSArraydb folder on the specified drive.

Table 1-2 Files located in the BDDatabase folder

File	Function
BDFACS.db	Database file
BDFACS.log	Database log file
BDData	Folder for storing BD FACSArray list mode data
dbvalidate.log	Log file created during backup procedure

2

Software Overview

This chapter provides an overview of the BD FACSArray system software components that you will use to create and run your experiments. For specific examples on how these components are used, see Creating a BD CBA Experiment Using the Wizard on page 169 and Creating an Experiment Using the Wizard on page 204.

The following topics are covered in this chapter:

- Software Startup on page 40
- Workspaces on page 43
- Workspace Components on page 52
- Software Shutdown on page 87

Software Startup

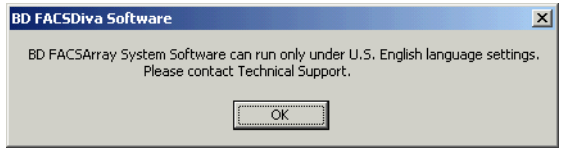
Before starting the software for the first time, review the BD FACSArray ReadMe file. A shortcut is copied to the Windows desktop during installation.

1 To start up the software, do one of the following:

- Double-click the shortcut icon on the desktop.
- Choose Start > Programs > BD FACSArray Software > BD FACSArray System Software.

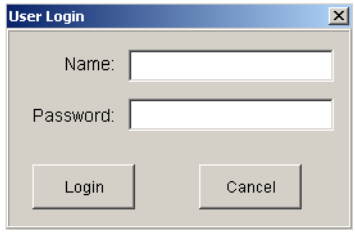
shortcut icon on desktop

2 If your operating system is non-US–English, the following message appears.


BD FACSDiva Software
BD FACSArray System Software can run only under U.S. English language settings.
Please contact Technical Support.
OK

See the Notice on page 30 for more information.

3 The User Login dialog appears.



4 Enter your user name and password.

NOTICE You can have a blank password.

5 Click Login.

- If login fails, a message appears asking you to re-enter your user name and password.

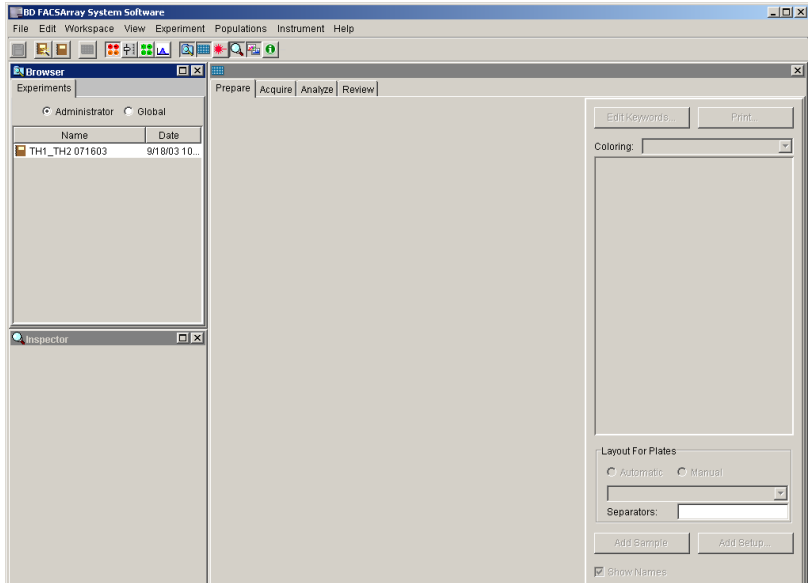
  NOTICE User names and passwords are case-sensitive.

  If log in fails three times, the software quits.

- If login is successful, the BD FACSArray System Software splash screen appears.

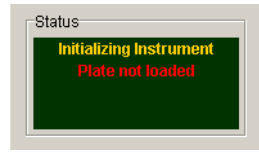
Once login is complete, the splash screen disappears and the Prepare workspace appears (Figure 2-1 on page 42). For a description of the different workspaces and workspace components, see Workspaces on page 43 andWorkspace Components on page 52.

Figure 2-1 Default Prepare workspace



When you launch the software, the system goes through a series of checks to verify that the bioanalyzer and computer are working properly. During this time, the Status box (in the Acquire tab of the Plate Editor) shows *Instrument not connected* and the LED indicator on the front of the instrument turns amber.

The system performs a homing sequence and begins initializing the fluidics. During this time the Status box shows *Initializing Instrument*.



⚠ **NOTICE** To avoid damaging the bioanalyzer, do not quit the software while the fluidics are initializing.

Once fluidics initialization is complete, (this process takes approximately 7 minutes), the Status box shows *Instrument ready* and the LED indicator on the front of the instrument turns green.

If there is a problem with the system, the LED indicator, located on the upper front panel of the instrument, turns amber or red. See LED Indicator on page 100 for troubleshooting information.

Workspaces

BD FACSArray system software is organized into four different workspace views designed to streamline experiment preparation, setup, acquisition, and analysis. When you launch BD FACSArray system software, the Prepare *workspace* opens by default.

Frames containing specific application components are displayed within each workspace. You can show, hide, resize, and reposition the frames within each workspace. See Workspace View Options on page 51. See Figure 2-8 on page 53 to view the application toolbar, where you access each workspace.

Prepare Workspace

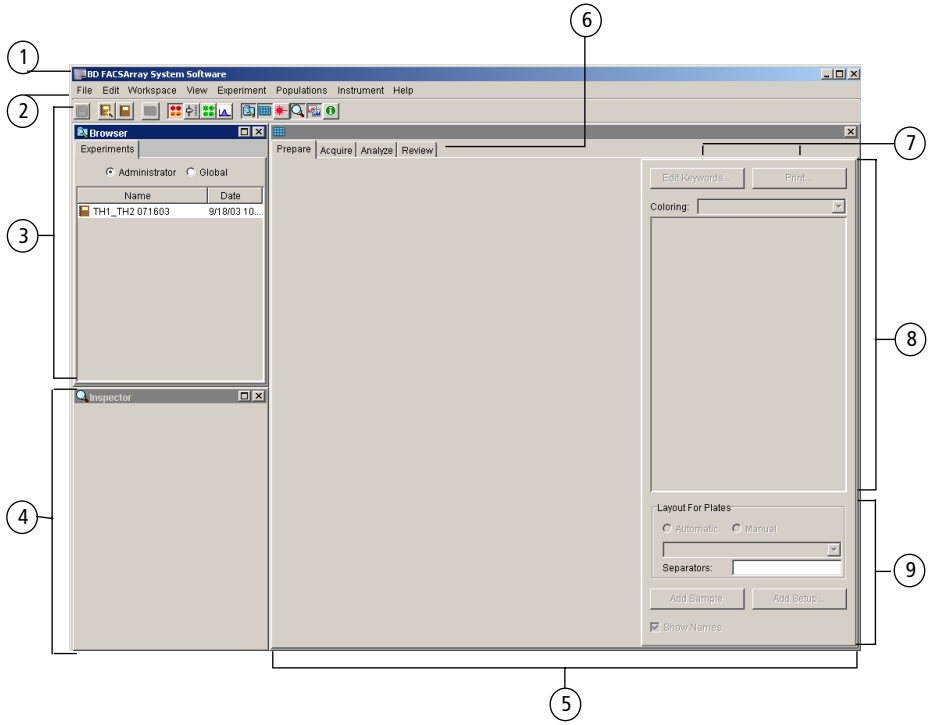
Open the Prepare workspace by clicking the Prepare tool ( ) in the application toolbar. You can also open the workspace by choosing it from the Workspace menu.

This workspace is typically used for the following operations.

- Creating a new experiment
- Modifying an existing experiment

Figure 2-2 on page 44 shows how the default Prepare workspace looks when you start the software. The figure legend shows the elements and frames located within the Prepare workspace and in the Plate Editor frame. A more detailed description of each element or frame begins on page 52.

Figure 2-2 Prepare workspace



Workspace Elements

1. Menu bar
2. Application toolbar
3. Browser
4. Inspector
5. Plate Editor

Plate Editor Elements

6. Plate Editor tabs
7. Edit Keywords/ Print buttons
8. Coloring Control group
9. Layout for Plates Control group

Setup Workspace

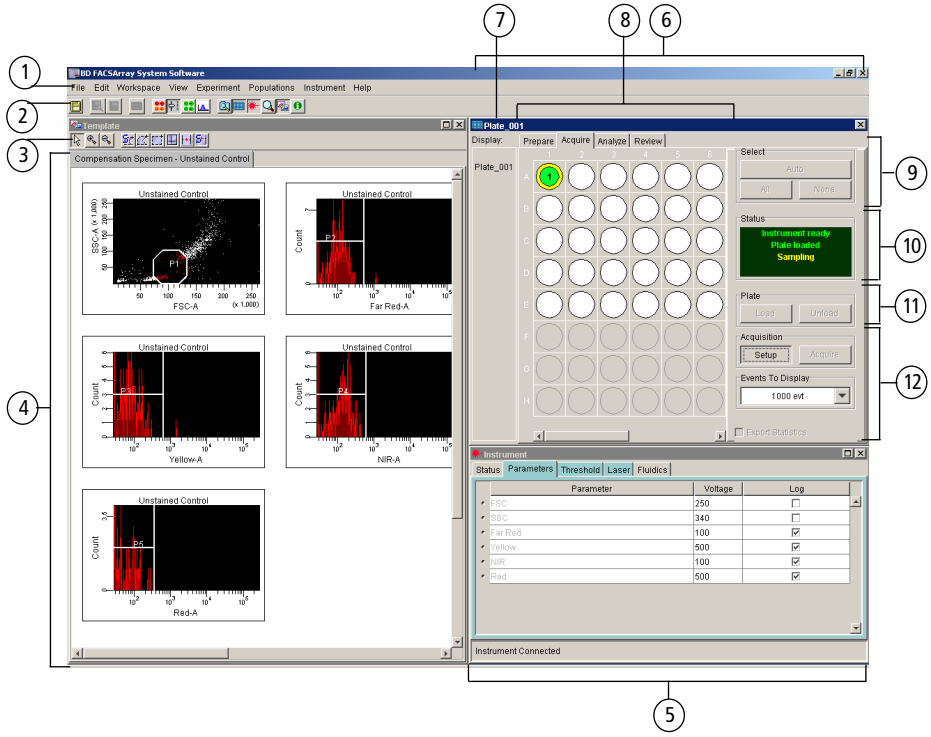
Open the Setup workspace by clicking the Setup tool ( ) in the application toolbar. You can also open the workspace by choosing it from the Workspace menu.

This workspace is typically used for the following operations.

- Running in Setup mode while viewing data
- Optimizing instrument settings while viewing data

Figure 2-3 on page 46 shows the Setup workspace for the 4-Color experiment template during optimization of the unstained control in a four-color immunophenotyping experiment. The figure legend shows the elements and frames located within the Setup workspace and in the Plate Editor frame. A more detailed description of each element or frame begins on page 52.

Figure 2-3 Setup workspace



Workspace Elements

1. Menu bar
2. Application toolbar
3. Template toolbar
4. Template view
5. Instrument frame
6. Plate Editor

Plate Editor Elements

7. Display list
8. Plate view
9. Select Control group
10. Status box
11. Plate Control group
12. Acquisition Control group

Acquire Workspace

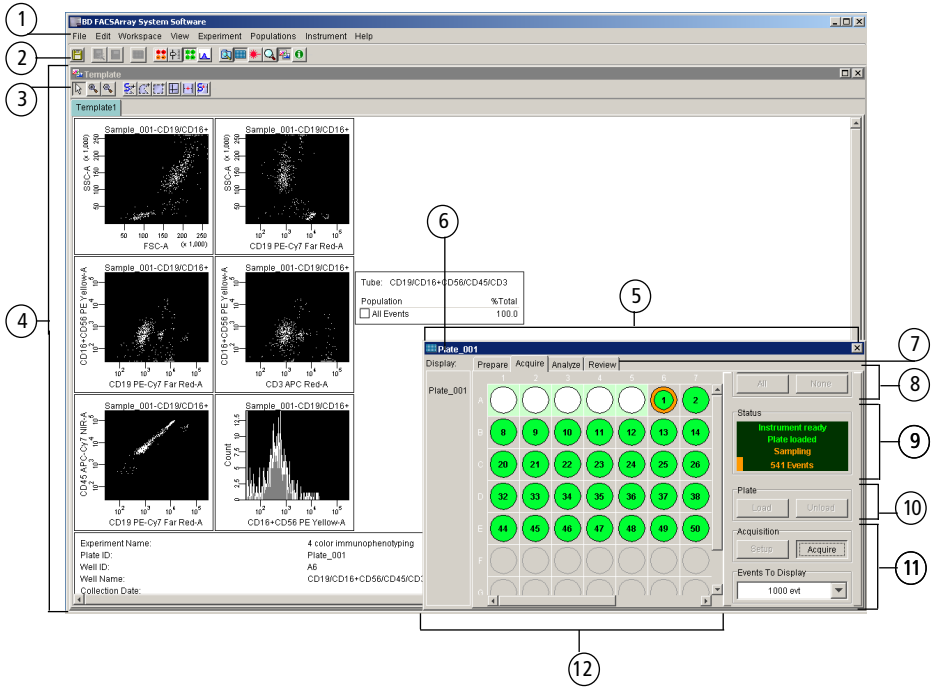
Open the Acquire workspace by clicking the Acquire tool ( ) in the application toolbar. You can also open the workspace by choosing it from the Workspace menu.

This workspace is typically used for the following operations.

- Acquiring and monitoring data
- Viewing acquisition status

Figure 2-4 on page 48 shows the Acquire workspace for the 4-Color experiment template. The figure legend shows the elements and frames located within the Acquire workspace and in the Plate Editor frame. A more detailed description of each element or frame begins on page 52.

Figure 2-4 Acquire workspace



Workspace Elements

1. Menu bar
2. Application toolbar
3. Template toolbar
4. Template view
5. Plate Editor

Plate Editor Elements

6. Display list
7. Plate Editor tabs
8. Select Control
9. Status box
10. Plate Control group
11. Acquisition Control group
12. Plate view

Analyze Workspace

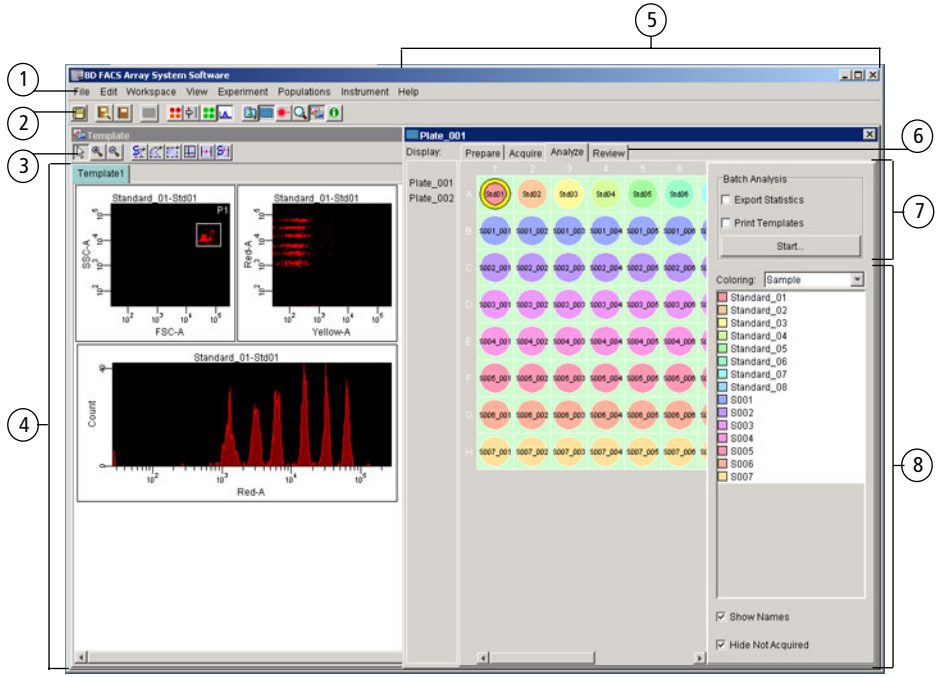
Open the Analyze workspace by clicking the Analyze tool ( ) in the application toolbar. You can also open the workspace by choosing it from the Workspace menu.

This workspace is typically used for the following operations.

- Analyzing data of individual wells
- Analyzing data of entire experiment or plate in batch mode
- Viewing data that has already been analyzed

Figure 2-5 on page 50 shows the Analyze workspace while viewing CBA data. The figure legend shows the elements and frames located within the Analyze workspace. A more detailed description of each element or frame begins on page 52.

Figure 2-5 Analyze workspace



Workspace Elements

1. Menu bar
2. Application toolbar
3. Template toolbar
4. Template view
5. Plate Editor

Plate Editor Elements

6. Plate Editor tabs
7. Batch Analysis Control group
8. Coloring Control group

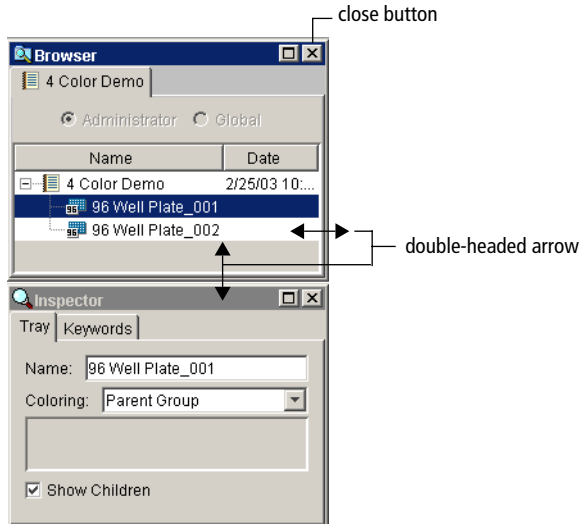
Workspace View Options

To maximize the workspace, click the maximize button. The workspace will expand to fill the screen.

Figure 2-6 Location of maximize button

- To move a frame, drag the title bar to a new position on the screen.
- To resize a frame, position the cursor on the border of the frame. When the cursor changes to a double-headed arrow, drag the border in the direction of the arrow.
- To collapse or expand a frame, click on the double-headed arrow in the border between the two components.

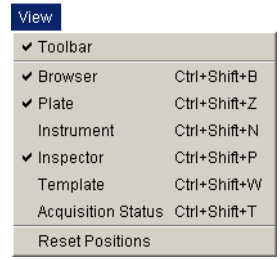
Figure 2-7 Resizing a workspace frame



NOTICE You can only move, resize, expand, and collapse the Acquisition Status, Browser, Inspector, Plate Editor, Instrument, and Template view frames.

- To view or hide different frames in the workspace, choose an option from the View menu, or click the corresponding tool in the application toolbar. See Application Toolbar on page 53 for a description of each tool.

  You can also hide a frame by clicking the close button (Figure 2-7 on page 51).



☑ **Tip** If you open a frame and it does not appear in the workspace, reduce the size of the Plate Editor.

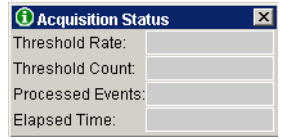
- To restore frames to their default position and size, choose View > Reset Positions.

  NOTICE If you resize or move frames, the new settings become the default position and size. You can restore frames to the software default positions by choosing View > Reset Positions.

Workspace Components

Acquisition Status Frame

Use the Acquisition Status frame to display and monitor Threshold Rate, Threshold Count, Processed Events, and Elapsed Time during sample acquisition. Open the Acquisition Status frame by clicking the Acquisition Status tool ( ) in the application toolbar.



Threshold Rate—The rate of events/sec that are above threshold and are detected by the bioanalyzer.

Threshold Count—The number of events that are above threshold and are detected by the bioanalyzer.

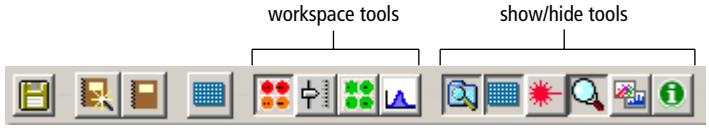
Processed Events—The number of events that are processed by the computer.

Elapsed Time—The amount of time elapsed since clicking Setup or Acquire.

⚠ **NOTICE** Depending on system resources and sample characteristics, there will be a slight discrepancy between Processed Events and Threshold Count.

Application Toolbar

Figure 2-8 Application toolbar



The Application toolbar contains the following tools.

Save—saves the current experiment to the database. Experiments are also saved when you close an experiment or quit the application.

Experiment Wizard—opens the Experiment Wizard. See page 204.

New experiment—opens the Template Selection dialog. See Creating New Experiments on page 56.

New plate—adds a new plate to the open experiment.

Prepare—opens the Prepare workspace. See page 43.

Setup—opens the Setup workspace. See page 45.

Acquire—opens the Acquire workspace. See page 47.

Analyze—opens the Analyze workspace. See page 49.

Browser—shows or hides (by clicking) the Browser. See page 54.

Plate—shows or hides (by clicking) the plate view. See page 84.

Instrument—shows or hides (by clicking) the Instrument frame. See page 66.

Inspector—shows or hides (by clicking) the Inspector frame. See page 60.

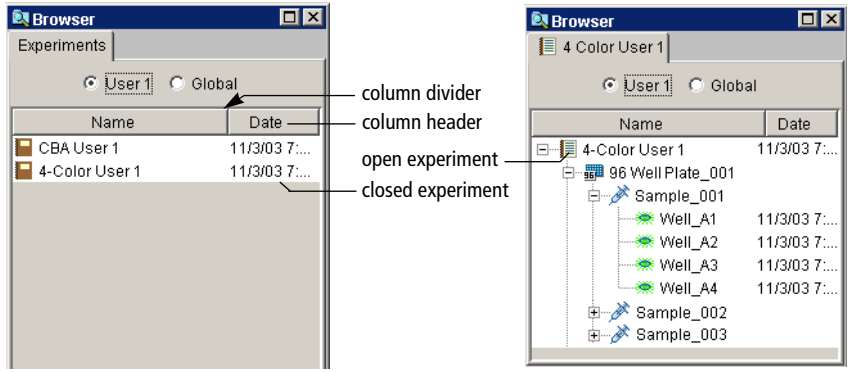
Template—shows or hides (by clicking) the Template view. See page 86.

Acquisition Status—shows or hides (by clicking) the Acquisition Status frame. See page 52.

Browser

The Browser lists all experimental data in a hierarchical view. Display the Browser by clicking the Browser tool ( ) in the application toolbar. When BD FACSArray software launches, the Browser displays experiments in a collapsed (closed) state. When you open an experiment, the Browser shows only the elements in that experiment. When you close the experiment, the Browser again shows all of your experiments in the collapsed state. See Figure 2-9.

Figure 2-9 Browser showing closed (left) and open (right) experiment



User name radio button—when selected, lists in the Browser only your experiments.

NOTICE The user name in Figure 2-9 is *User 1*.

Global radio button—when selected, lists in the Browser your experiments and any shared experiments.

Table 2-1 Browser Elements

	Open experiment—lists the plates in each experiment, the samples on each plate, and the wells selected for each sample
	Closed experiment—lists the experiment name
	Shared experiment—indicates that an experiment is shared with other users
	Plate—lists the samples on each plate, and the wells selected for each sample
	Sample—lists the wells selected for each sample
	Empty well—indicates a well without data
	Well with data—indicates a well that contains data

Right-click any item in the Browser to display a contextual menu with application options specific to that item. A summary of all contextual menus is provided in Appendix A on page 355.

The Browser has the following functions.

- lists your saved experiments, and those shared, in the BDFACS database.

  Browser elements can be listed by name or date in ascending or descending order.

⚠ **NOTICE** Adding or deleting elements from the Browser will add or delete elements from the database. All associated FSC files will also be deleted from the hard drive.

- provides an interface for setting up experiments

  You must click on elements in the Browser to activate certain tools. For example, you must click on a plate to open the Plate Inspector.

- organizes experimental elements in a hierarchical view

  View elements listed under an experiment, plate, sample, or well by clicking once on the plus sign (+) next to the corresponding icon.

  - Sort experiments in the Browser by double-clicking inside a column header. Double-click in the same header to reverse the sort order. A downward arrow indicating ascending sort order or an upward arrow indicating descending sort order appears in the column.

  - Resize columns in the Browser by dragging the column dividers.

- provides shortcuts for renaming database elements

  Rename any Browser element in an open experiment by selecting the element and entering a new name. (Alternatively, you can select the item and choose Edit > Rename, or right-click the item and choose Rename.) A blinking cursor appears indicating the field is editable.

⚠ **NOTICE** When you rename a Browser element, the contents of any associated FCS files remain unchanged.

Experiments

An Experiment is a group of elements used to acquire and analyze data from the bioanalyzer. Experiments are organized hierarchically into plates, samples (material to be analyzed), wells (acquisition data and reagents used to analyze the sample), instrument settings (if a well has been acquired), and analysis data (plots, gates, and statistics).

Creating New Experiments

You can create experiments automatically using the Experiment Wizard or manually.

☑ **Tip** BD recommends using the Experiment Wizard when possible to create new experiments because it greatly simplifies experiment setup.

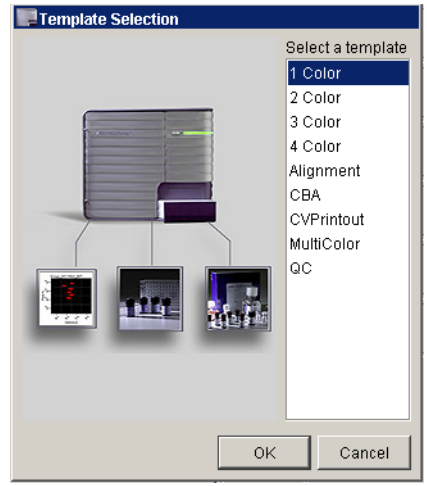
For tutorials on setting up experiments using the Experiment Wizard, see page 169 to create a CBA experiment or page 204 to create an immunophenotyping experiment.

For a tutorial on setting up a simple experiment manually, see page 238.

To create a new experiment, click the New Experiment tool ( ) in the application toolbar or choose Experiment > New Experiment (Ctrl-E).

The Template Selection dialog opens. Choose an experiment template and then click OK.

The currently opened experiment closes and a new, open experiment is added to the Browser. A new experiment consists of a single plate.



Opening Experiments

NOTICE You can edit elements and acquire data only within an open experiment, and only one experiment can be open at a time. An open experiment is indicated by an open-book icon ( ). You cannot close an open experiment during acquisition.

To open a closed experiment:

- Double-click a closed experiment icon in the Browser ( ), or
- Select an experiment in the Browser and choose Experiment > Open Experiment (Ctrl-O), or
- Right-click an experiment icon in the Browser and choose Open Experiment.

There might be a short delay while the software retrieves the experiment from the database.

Saving Experiments

Any changes to an open experiment are saved when you close the experiment, quit the application, or click the Save tool in the application toolbar ( ). A wait cursor is displayed while the experiment is saving. FCS file data, instrument settings, and keywords are saved after a well is successfully saved. (A green icon next to the well ( ) indicates data has been saved.) The Experiment modification date is automatically updated each time data in the experiment changes.

☑ **Tip** Locate saved data more easily by naming experiments and experiment elements with meaningful names.

To save an open experiment as a template, choose Experiment > Save As Template.

The experiment is saved in the Templates folder. It retains the same name as the original experiment. The new experiment retains all gates, instrument settings, and loader settings as the original experiment. If setup wells were run, the template also saves the optical spillover matrix.

Access the new experiment template by choosing Experiment > New Experiment and selecting it from the template list. You can also access the new experiment template by choosing it in the Template view of the Experiment Wizard.

Designating Experiments as Shared or Private

You can designate your experiments as being shared or private.

- Sharing experiments allows other users to view and use your experiments. However, other users can also alter and delete your shared experiments.

  To share your experiments, do the following:

  - Right-click the experiment icon (closed or open) in the Browser.
  - Choose Share Experiment.

    The experiment icon changes to a shared experiment icon ( ).

- Designating experiments as private will prevent other users from viewing or using your experiments. New experiments are designated as private by default.

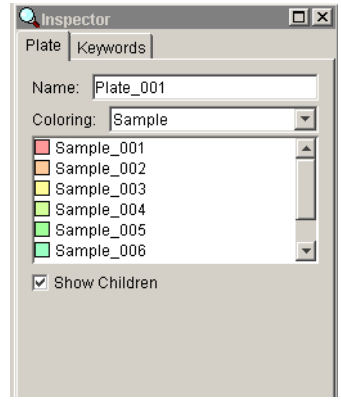
  To designate your experiments as private, do the following:

  - Right-click the experiment icon (closed or open) in the Browser.
  - Choose Make Private.

    The experiment icon remains unchanged.

Plates

A plate consists of a list of samples and a list of wells used to analyze the samples.



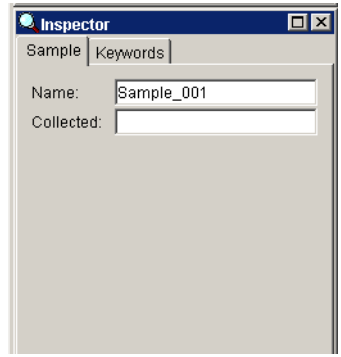
Use the Plate Inspector to enter or change the plate name, choose the coloring-coding of the wells in the plate view, choose whether to show in the Browser the samples and wells listed within the plate, and view the keywords defined for the plate.

NOTICE Plate names cannot contain periods or commas. Spaces at the beginning or end of the name are automatically removed.

Samples

A sample consists of the name of the material to be analyzed and a list of the wells used to analyze the material.

Use the Sample Inspector to enter the sample name and collection date of material in the wells. To enter keywords that will be stored with the Sample, see Keywords on page 61.



NOTICE Sample names cannot contain periods or commas. Spaces at the beginning or end of the name are automatically removed.

To add samples to an experiment, see page 240.

Wells

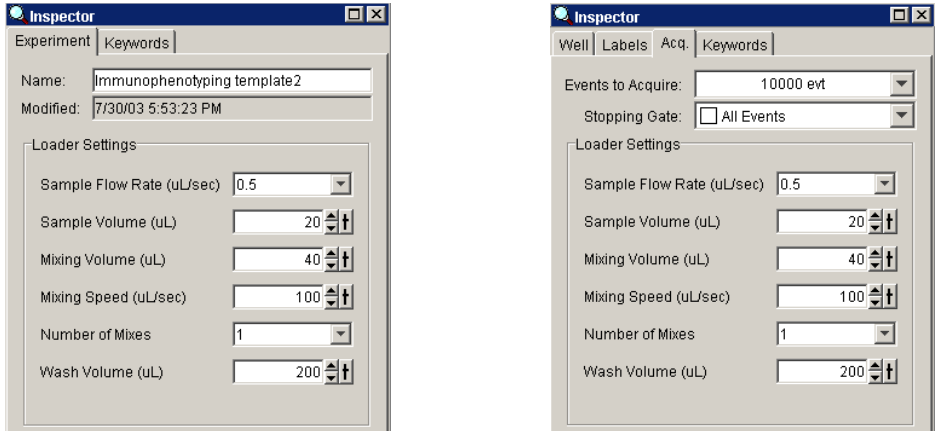
A well can contain acquisition criteria, information about the reagents used to analyze the sample, the data for saved events, and instrument setting adjustments made for that well. Keywords can also be saved with well data. Most well-specific information is entered using the Well Inspector. See Figure 2-10 on page 61.

Inspector

The Inspector provides an easy-to-use interface for viewing or modifying the attributes of a single object or set of objects in the Template view, Plate Editor, or Browser. For example, you can use the Inspector to view and edit keywords, change plate attributes like the well color and whether to show sub-populations, or enter the name of an experiment, sample, or well. There is an Inspector for the following elements: experiment, plate, sample, well, template, plot, and statistics.

To display the Inspector frame, click the Inspector tool ( ) in the application toolbar. The contents of the Inspector frame vary depending on the object selected. For example, Figure 2-10 on page 61 compares the contents of an Experiment Inspector (displayed when an Experiment is selected in the Browser) with those of a well Inspector (displayed when a well is selected on the plate view).

Figure 2-10 Experiment Inspector (left) vs well Inspector (right)



Keywords

Keywords can be defined and saved in the database for experiments, plates, samples, and wells. Only well keywords are listed in the header of exported FCS data files.

Use keywords for the following:

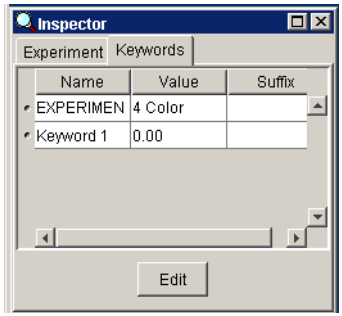
- Define a list of terms (Selectable Strings) that can be stored with each experiment. Attach numerical data, such as cell count, to a well or sample.
- Attach labels to data.
- Display well and sample keywords in the headers of statistics views. Keywords are exported along with the statistics.

Defining Keywords at the Experiment, Plate, or Sample Level

You can define keywords at the experiment, plate, or sample level by using the Keywords tab in the Inspector.

1 In an open experiment, select an experiment, plate, or sample in the Browser.

2 Click the Keywords tab in the Inspector of the selected item.

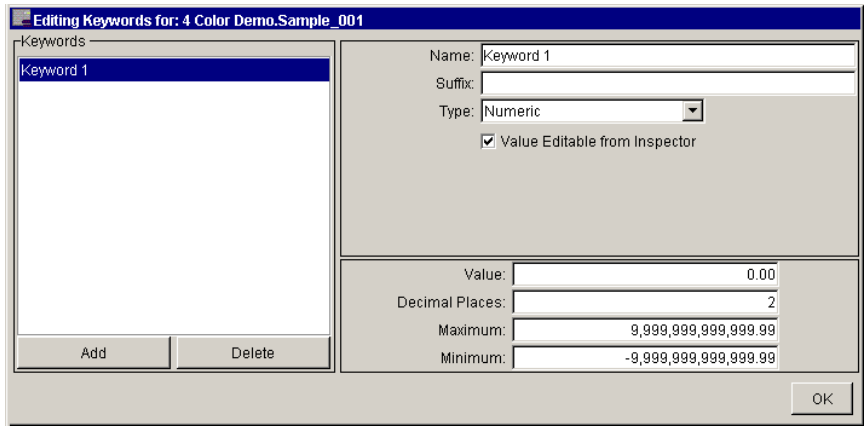


3 Click the Edit button to add or change a keyword.

The Keyword Editor appears where you can add, define, or delete keywords.

4 Click the Add button to create a keyword.

Figure 2-11 Keyword Editor



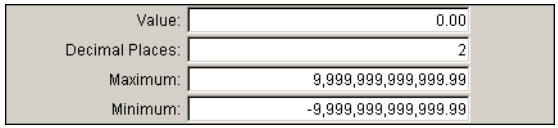
5 Name the keyword and add any required suffix.

Each keyword name must be unique; use the suffix to define values, such as units of measure.

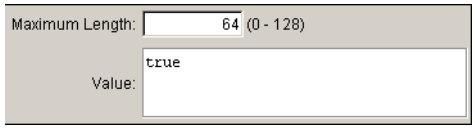
6 Select the type of keyword.

You can specify a Value in the dialog box or check *Value Editable from Inspector* and enter the value in the Inspector.

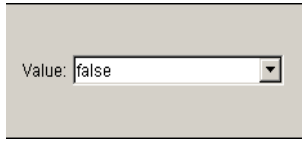
- Use *Numeric* for keywords defined by numerical values, such as numbers of cells. Limit the range by entering Minimum and Maximum values; enter a number to specify the number of digits to the right of the decimal place (maximum of 14).



- Use *String* for keywords defined by text, such as sample identifiers. You can limit the Maximum Length to no more than 128 characters. The default length is 64 characters. Changing the maximum length to a smaller value truncates the string.

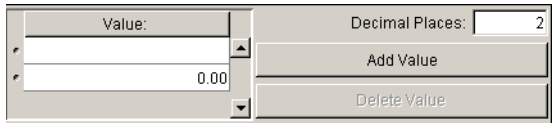


- Use *Boolean* for keywords that require a true or false answer. False is selected as the default.

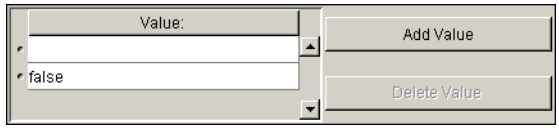


- Use *Selectable Numeric* to define a set of selectable numeric keywords, such as a list of values (maximum of 20). Define the set of values by clicking Add Value, selecting the value in the Value field, and entering the required value. All defined values will appear in a drop-down menu in the Value field of the Keyword Inspector.

Use the Decimal Places field to specify the number of digits to the right of the decimal place (maximum of 14). The default number of decimal places is 2.



- Use *Selectable String* to define a set of selectable text keywords, such as operator names. Define the set of selections by clicking Add Value, selecting the value in the Value field, and entering the required text. (The first value is a non-value that cannot be changed.) All defined values will appear in a drop-down menu in the Value field of the Keywords Inspector.



7 Click OK to dismiss the Keyword Editor.

Defining Keywords at the Well Level

You can edit keywords for all wells in the experiment by using the Edit Keywords button in the Prepare tab of the Plate Editor; see Edit Keywords Button on page 73.

1 In the plate view, select one or more well(s).

2 Click the Edit Keywords button in the Prepare tab of the Plate Editor to access the Keyword Editor (Figure 2-11 on page 62).

3 Repeat step 4 on page 62 through step 6 on page 63 in Defining Keywords at the Experiment, Plate, or Sample Level.

4 Click OK to dismiss the Keyword Editor.

5 If needed, edit Keyword values by selecting the wells in the plate view and then entering a new value in the Well Inspector.

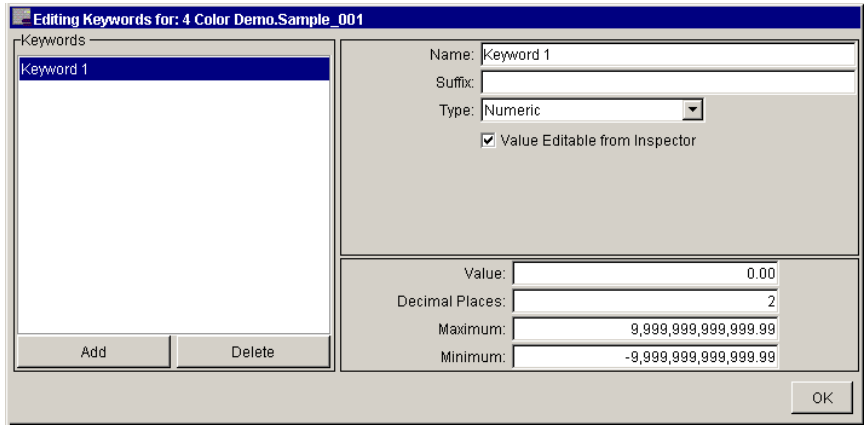
Deleting Keywords at the Experiment, Plate, or Sample Level

1. In an open experiment, select an experiment, plate, or sample in the Browser.

2. Click the Keywords tab in the Inspector of the selected item.

3. Click the Edit button to access the Keyword Editor.

Figure 2-12 Keyword Editor



4. Select a keyword from the list, and click Delete.

Deleting Keywords at the Well Level

NOTICE Keywords can also be deleted by clicking Edit Keywords in the Prepare tab of the plate view.

1. In the plate view, select one or more well(s).

2. Click the Edit Keywords button in the Prepare tab of the Plate Editor to access the Keyword Editor (Figure 2-12).

3. Select a keyword from the list, and click Delete.

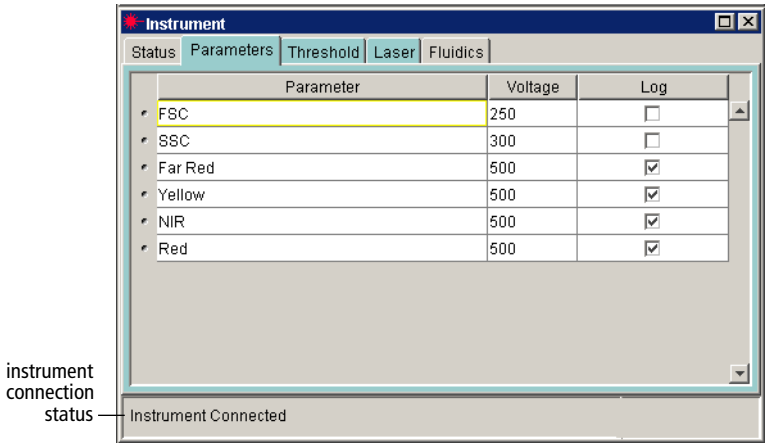
Instrument Frame

When an experiment is open in the Browser, the Instrument frame has five tabs: Status, Parameters, Threshold, Lasers, and Fluidics. By clicking the tabs, you can view error messages, adjust instrument settings, set one or two threshold values, monitor laser status and power, and monitor fluidic levels and pressure.

NOTICE Before opening an experiment, the Instrument frame contains only the Status, Laser, and Fluidics tabs.

The Instrument frame is located in the Acquire tab of the Plate Editor. However, you can view the Instrument frame in any workspace by clicking the Instrument tool ( ) in the Application toolbar.

Figure 2-13 Instrument frame located in the Acquire tab of the Plate Editor

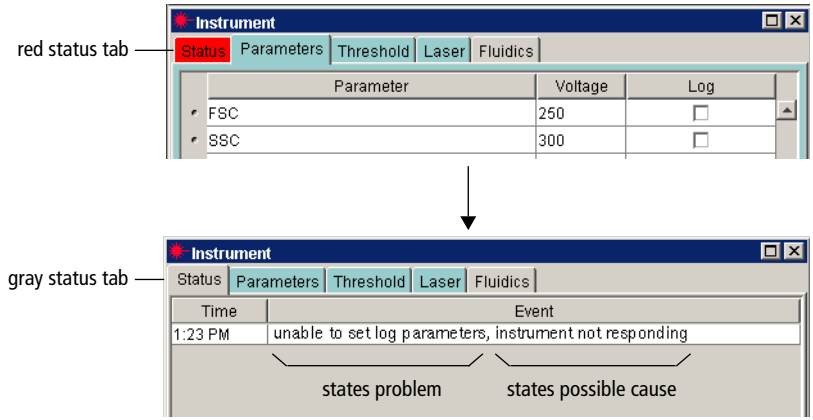


Status Tab

If there is a problem with the instrument, the Status tab of the Instrument frame will display detailed error messages (in the Event column) and the time (in the Time column) that the errors occurred. See Figure 2-14 on page 67.

If an instrument error occurs and the Status tab is not selected, the color of the Status tab will turn red to alert you to the problem. Once you click the Status tab, the tab color will return to gray. To clear displayed error messages, click the Clear button. For troubleshooting information, see page 337.

Figure 2-14 Status tab of Instrument frame



Parameters Tab

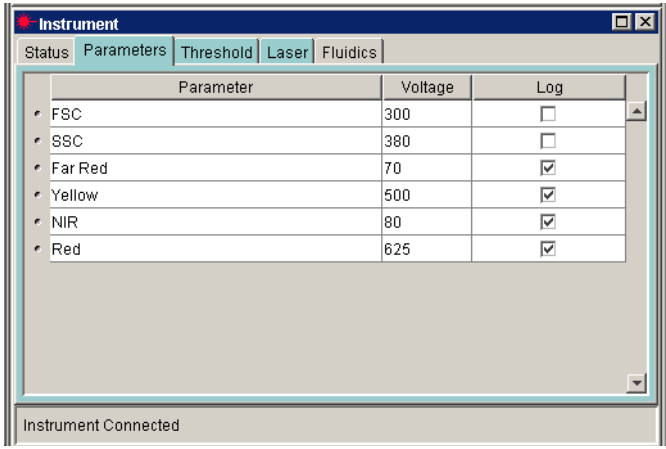
Use the Parameters tab of the Instrument frame (Figure 2-15 on page 68) to set and adjust the parameter voltages and choose whether data will be collected in linear or log mode.

Adjusting voltage while acquiring a sample causes sample populations to move either up and down, or right and left. To adjust parameter voltage, change the value by using the up or down arrow, the slider bar, or entering a value in the field.

- Enter a number in the Voltage field ranging from 1 through 1,000; press the Return key on the computer keyboard to save the value.
- Click in the Voltage field and use the up and down arrows to change values. Hold down the Ctrl-key while clicking the arrows to adjust values in increments of 10.
- Click in the Voltage field and then click on the slider control to change values. Hold down the Ctrl-key while clicking on the slider to adjust values in increments of 100.

Linear and log mode are two different ways to display data. Plots can display data in Linear or Log scale, or both. Statistics are not affected by the way the data is displayed, so choose whichever format is appropriate. Select the Log checkbox to save data in log mode. Deselect the checkbox to save data in linear mode. See Figure 4-1 on page 108 to view different plot types.

Figure 2-15 Parameters tab of Instrument frame



NOTICE There are default instrument settings for a limited number of assays. When you use the Experiment Wizard to create a new experiment, you will have the choice of using a CBA or cell-based (1-, 2-, 3-, 4-, or multicolor) template with default instrument settings. However, you might need to further optimize instrument settings. See Creating an Experiment Using the Wizard on page 204 for more information.

Threshold Tab

A threshold is a limit above which signals are collected to eliminate background noise. Because BD FACSArray data is digital, one or two thresholds can be set as numerical values including logical and/or expressions. A threshold can be set on any signal from any laser. Most immunophenotyping and cytometric bead array (CBA) experiments threshold on forward scatter (FSC). More advanced applications might benefit from setting threshold values on two different detectors to get better quality data.

Use the Threshold tab (Figure 2-16 on page 69) to add and delete threshold parameters, adjust threshold values to exclude unwanted events, and combine threshold parameters using the And/Or radio buttons.

- Enter a number in the Value field ranging from 200 through 262,143; press the Return key on the computer keyboard to save the value.

- Click in the Value field to open a slider control to adjust values in increments of 1,000; press the Return key on the computer keyboard to save the value.

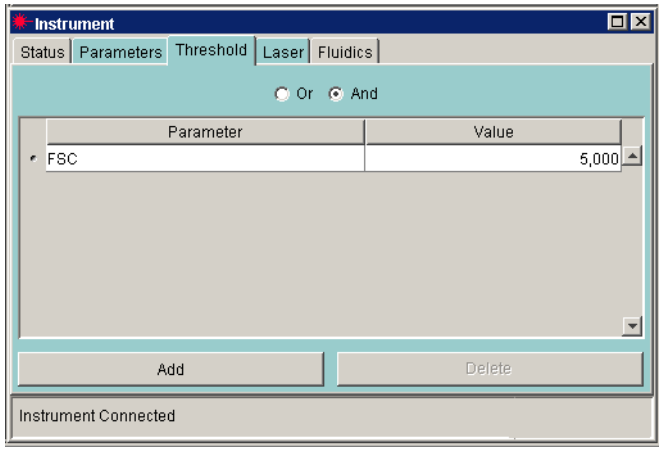
- Click in the Value field and hold down the Ctrl-key while moving the slider to adjust values in increments of 5,000. Press the Return key on the computer keyboard to save the value. The default value is FSC at 5,000.

- Create logical and/or expressions when combining two threshold values. The default is set to And.

  - Clicking the And radio button excludes all events that fall below the threshold value of both parameters.

  - Clicking the Or radio button excludes all events that fall below the threshold value of either parameters.

Figure 2-16 Threshold tab of Instrument frame



Laser Tab

Use the Laser tab (Figure 2-17 on page 70) to monitor the status and power of the green laser. You can also use this tab to turn on test pulses for troubleshooting purposes when told to do so by BD Biosciences.

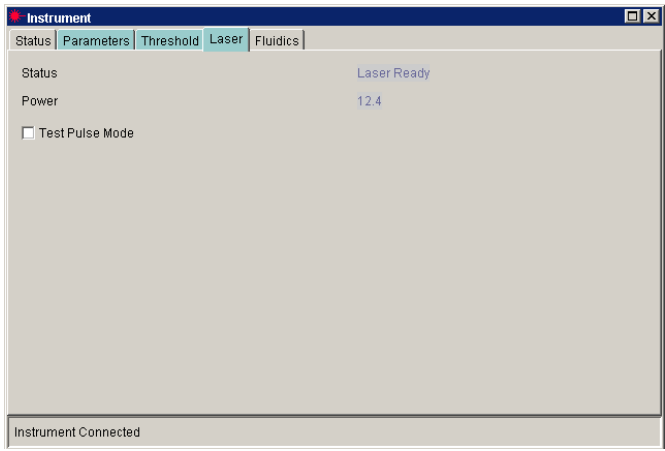
When you launch the software, the system performs a home sequence and begins initializing the fluidics. During this time, the Laser tab show laser status as Not Ready. Once fluidics initialization is complete, (this process takes approximately 7 minutes), the Laser tab shows the laser status as Ready. However, BD recommends allowing the laser to warm up for at least 20 minutes prior to running samples on the bioanalyzer.

⚠ **NOTICE** To ensure adequate laser power, do not acquire samples on the bioanalyzer until the laser has warmed up for at least 20 minutes.

If you are instructed by BD Biosciences to turn on test pulses, click to select the checkbox next to Test Pulse Mode. Once you are finished troubleshooting the bioanalyzer, be sure to deselect Test Pulse Mode.

⚠ **NOTICE** Because saving data while test pulses are turned on could negatively affect your data, be sure to deselect Test Pulse Mode when you are finished troubleshooting the bioanalyzer.

Figure 2-17 Laser tab of Instrument frame



Fluidics tab

Use the Fluidics tab to monitor the fluidics pressure.

Menu Bar

The following menus appear when you select one of the elements in the menu bar at the top of the workspace.

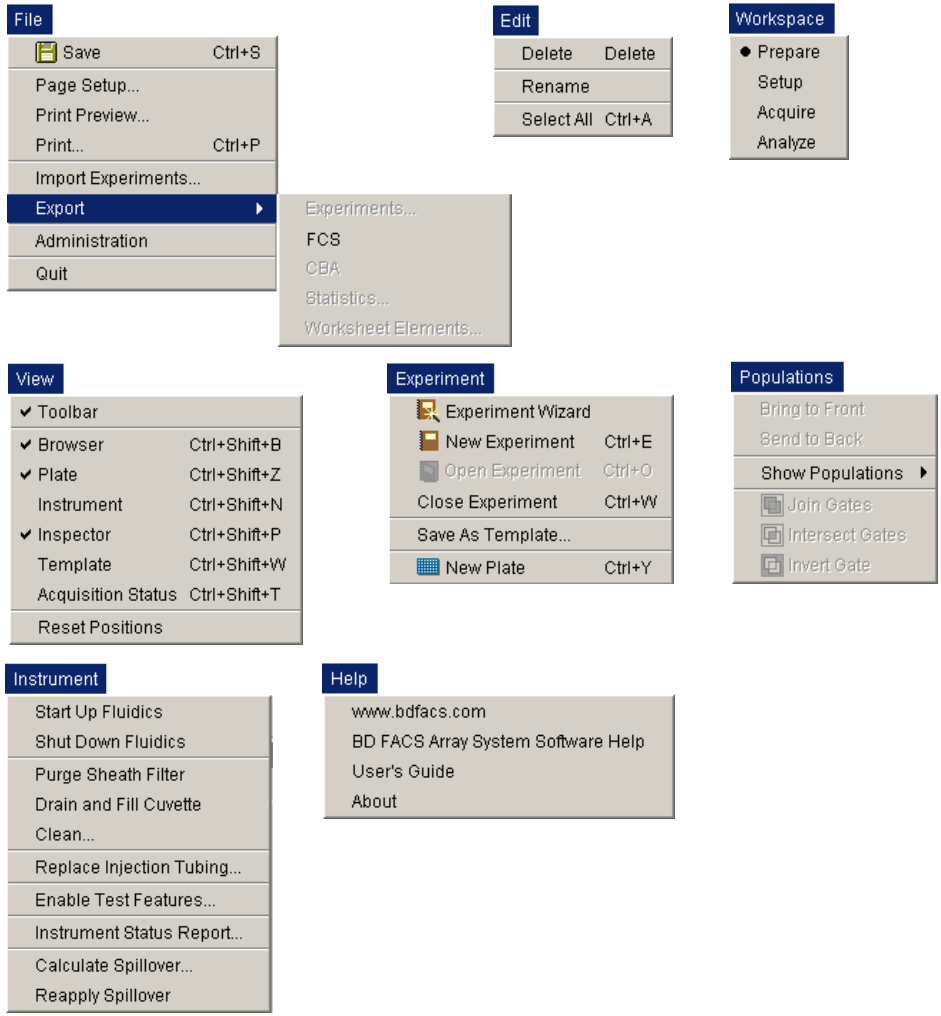


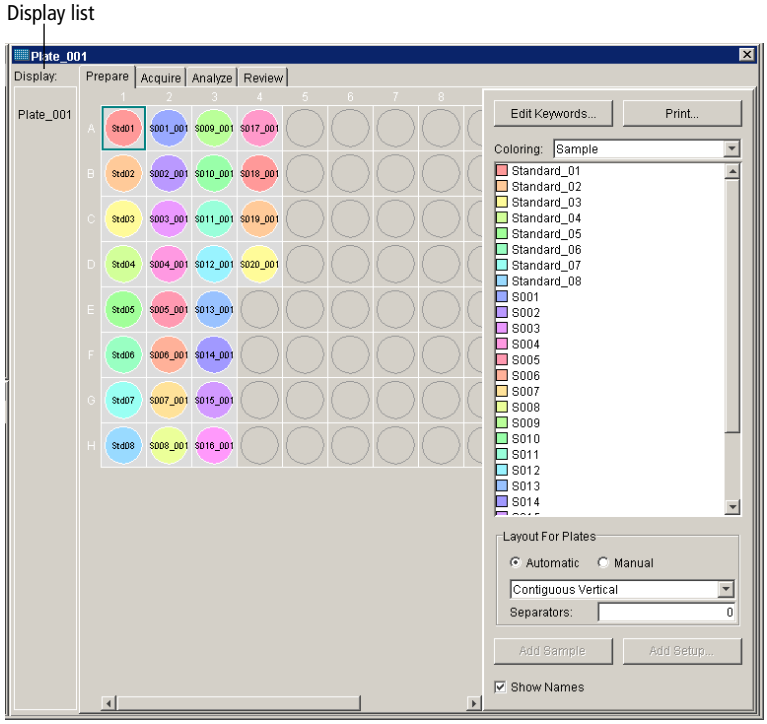
Plate Editor

There are four tabs in the Plate Editor. (See page 46 for location of the Plate Editor.)

Prepare tab

Use the Prepare tab of the Plate Editor to add samples to and arrange samples in your experiment, create new experiments, and modify existing experiments.

Figure 2-18 Prepare tab of Plate Editor showing Display list



Display List

The Display list (see Figure 2-18 on page 72) shows the plates defined in the open experiment. The number of plates displayed in the list depends on the number of samples in the experiment and the plate layout pattern selected in the Layout For Plates Control group. Click a plate in the Display list to show a representation of the plate view in the Plate Editor.

Edit Keywords Button

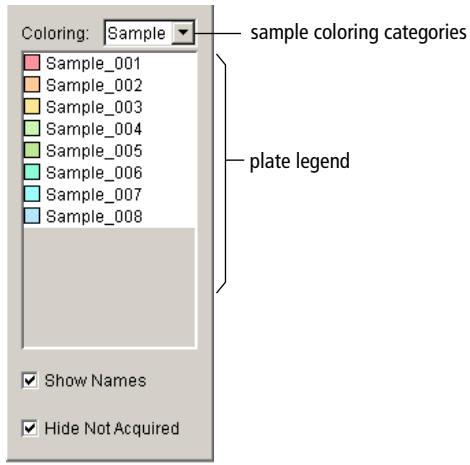
The Edit Keywords button brings up the Keyword Editor where you can add new keywords and delete or modify existing keywords within an experiment. For more information, see Keywords on page 61.

Print Button

Use the Print button to print a copy of the plate view. Pressing the Print button brings up a Page Setup dialog where you can choose different print orientations.

Coloring Control group

The Coloring Control group contains the Coloring drop-down menu, plate legend, and Show Names and Hide Not Acquired checkboxes.



- The Coloring drop-down menu allows you to choose between different color-coding categories. What you choose in the Coloring drop-down menu determines how the samples on the plate view are colored.

  - Sample category—Samples are automatically assigned a color based on sample name. Wells from the same sample are the same color.

  - Keyword category—Samples are colored based on the selected keyword value. Wells with the same selected keyword are the same color.

  NOTICE You cannot assign or change the color of individual samples. Samples are automatically colored depending on the coloring category you choose.

- The plate legend displays the color-coding of each sample. The legend is only for viewing.

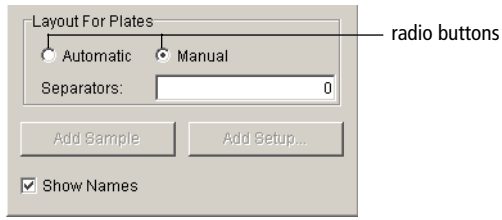
- *Show Names* checkbox—To display well names in the plate view. Deselect the checkbox to hide the well names.

- *Hide Not Acquired* checkbox—To hide wells that do not contain data. Deselect the checkbox to show all wells containing samples, whether or not they contain data. Hiding the wells that were not acquired (do not contain data) is the default.

Layout for Plates Control Group

The Layout for Plates Control group provides options for adding samples automatically or manually to an experiment. Elements within the group are either enabled or disabled depending on which option you choose.

- Manual Layout—To manually add samples to the plate view.



After you click the Manual radio button, the following elements become enabled in the Layout For Plates Control group.

- *Add Sample* button—allows you to add samples to the plate view after selecting the plate.
- *Add Setup* button—allows you to add setup wells to your plate.
- *Show Names* checkbox—allows you to show or hide the sample names in the plate view.

NOTICE Once you select the Manual radio button, the Automatic radio button becomes disabled and you cannot select it again for that experiment.

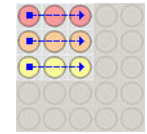
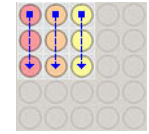
- Automatic Layout

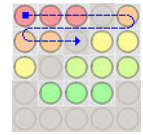
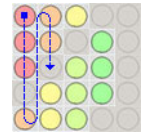
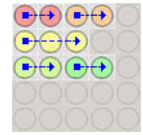
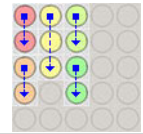
The *Automatic* radio button is used when creating experiments using the Experiment Wizard. For information on creating experiments using the Experiment Wizard, see page 204.

The following options are enabled in the Layout For Plates Control group.

- *Plate Layout* drop-down menu—allows you to select different patterns to arrange your samples on the plate.
- *Separators* field—allows you to enter the number of wells (0–5) you want between each sample.

The following list shows the different sampling patterns from which you can choose. In the figures, different colors indicate different samples.

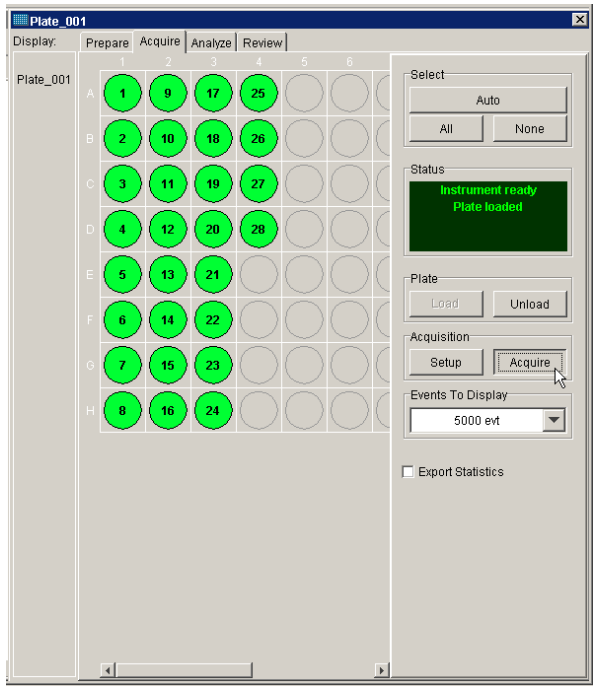
Sample per row	Sets up one sample per row. If a sample contains more than 12 wells, the sample continues to the next row.	
Sampler per column	Sets up one sampler per column. If a sample contains more than 8 wells, the sample continues to the next column.	

Contiguous horizontal	Sets up samples end to end, row by row. If a sample contains more than 12 wells, the sample continues to the next row. The layout shown to the right incorporates 1 separator.	
Contiguous vertical	Sets up samples end to end, column by column. If a sample contains more than 8 wells, the sample continues to the next column. The layout shown to the right incorporates 1 separator.	
Row-based	Sets up rows to contain as many complete samples as possible. If a sample does not fit on a row, a new row is started.	
Column-based	Sets up columns to contain as many complete samples as possible. If a sample does not fit on a column, a new column is started.	

⚠ **NOTICE** Once you acquire a well, you cannot change the layout pattern for that experiment.

Acquire Tab

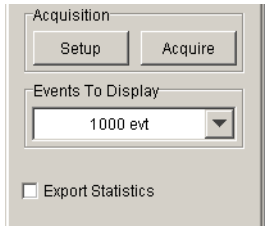
Use the Acquire tab of the Plate Editor to select the wells to acquire in the plate view, monitor the status of the instrument, load and unload a plate, run the instrument in setup mode to adjust instrument settings, acquire and save data, choose the number of events to display in the plots, and choose whether to export statistics.



Acquisition Control Group

The Acquisition Control group (Figure 2-19 on page 78) contains Setup and Acquire buttons for acquiring and saving events, a drop-down menu for choosing the number of events to display in plots in the Template view, and a checkbox to choose whether to export statistics.

Figure 2-19 Acquisition Control group



- *Setup* button—Enabled when a plate has been loaded into the instrument and at least one well has been selected for acquisition. Clicking the Setup button begins sampling from the first well and events appear in the plots, although data is not being saved.

  Use Setup to adjust instrument settings. Events will be acquired until a maximum acquisition time has been reached. This acquisition time is not adjustable and is calculated by the software for each sample based on sample volume. Press the Setup button again to stop acquisition.

- *Acquire* button—Enabled when a plate has been loaded into the instrument and at least one well has been selected for acquisition. Clicking the Acquire button begins acquisition of the first well; events appear in the plots, and data is saved.

  Events will be acquired until the specified number of events have been saved, or the maximum acquisition time has been reached. The maximum acquisition time is not adjustable and is calculated by the software for each sample based on sample volume. Press the Acquire button again to stop acquisition before the sample criteria have been met.

- *Events To Display* drop-down menu—For selecting the number of events to display in the plots.

☑ **Tip** While optimizing instrument settings, change the sample flow rate to 0.5 and choose a minimum number of events to display in the plots. Choosing the lowest sample flow rate allows more time to optimize settings and the software updates the plots more quickly when showing fewer events.

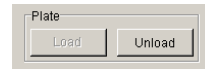
- *Export Statistics* checkbox—For appending the statistics results from the template to the statistics results file when acquisition is complete.

Plate Control Group

Use the Plate Control group to load and unload the multiwell plate. These buttons are enabled only when the computer is connected to the instrument and the status box shows that the instrument is ready.

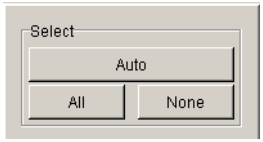
- The *Load* button is enabled when the Status box displays *Plate not loaded*.

- The *Unload* button is enabled when events are not being acquired and the Status box displays *Plate loaded*.



Select Control Group

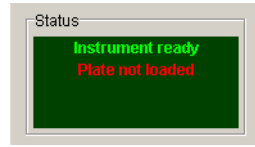
The Select Control group allows you to select wells in the plate view for acquisition.



- *Auto* button—click to select wells that contain sample and have not yet been acquired. This is the default setting when experiments have been created using the Experiment Wizard.
- *All* button—click to select all wells specified as sample or setup wells.
- *None* button—click to deselect wells that have been selected.

⚠ **NOTICE** Click on a well to toggle between selecting and deselecting the well.

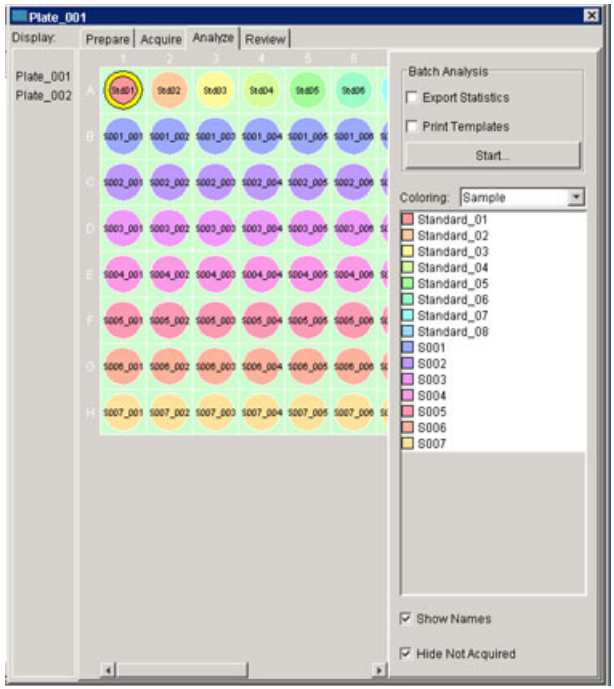
Status Box



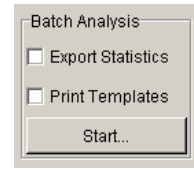
The Status box displays the status of the connection between the computer and the bioanalyzer, whether a plate has been loaded into the bioanalyzer, the status of acquisition, and any errors that occur during acquisition. If the instrument is ready for acquisition, the message *Instrument Ready* will be displayed on the Status box. For information on troubleshooting error messages, see Instrument Troubleshooting on page 338.

Analyze Tab

Use the Analyze tab of the Plate Editor to display acquired wells in the plate view, show the color-coding of the wells in the plate view, choose whether to display well names and wells not acquired, view the Coloring Legend, and choose options for and begin batch analysis.



Batch Analysis Control Group



Use the Batch Analysis Control group to choose whether to export analysis statistics, print the template, and start and cancel batch analysis of the wells containing data. The Start button is enabled if at least one well in the plate view contains data and one or both checkboxes are selected. Press the Cancel button in the Batch Analysis Control group to stop batch analysis.

- *Export Statistics* checkbox—To append the statistics results from the template to the statistics results file when analysis is complete.

  When you click Start, a file location dialog appears where you can enter the file name and choose where to save the file.

  - Press Save to begin batch analysis and save the export file.

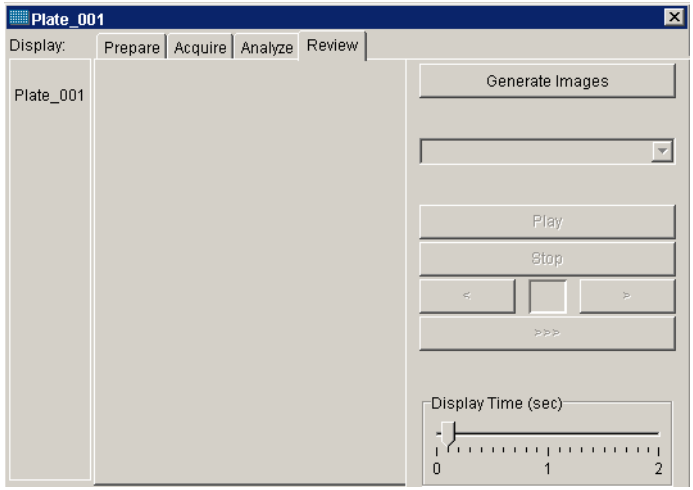
  - Press Cancel to begin batch analysis without saving an export file if you chose to print the templates, otherwise, batch analysis is aborted.

- *Print Template* checkbox—To print the Template view for each well after analysis.

Review Tab

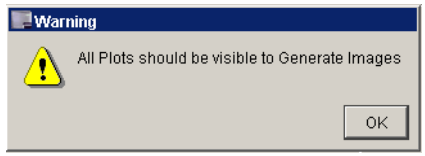
Use the Review tab to convert the results in the template view to a series of images of each plot analyzed (Figure 2-20 on page 82). You can review the images automatically or manually by using the forward and backward arrow buttons in the Review Control group. When you first click the Review tab, only the Generate Images button is enabled. Once images are generated, the drop-down menu and remaining buttons become enabled.

Figure 2-20 Review tab of Plate Editor



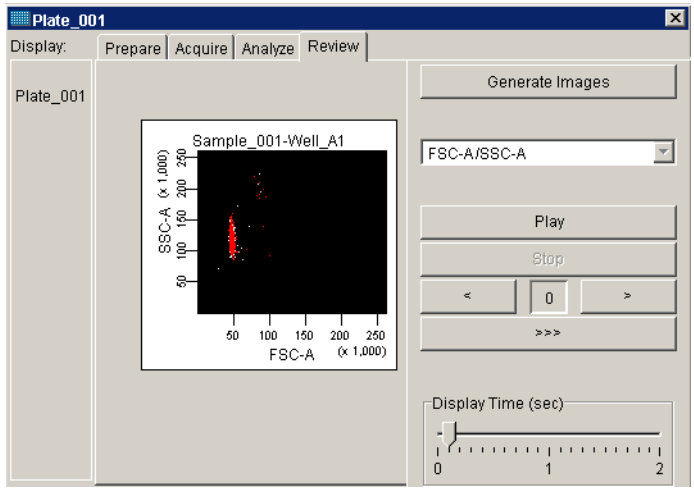
- *Generate Images* button—Before you click this button, verify the template view is displayed in the Review tab of the Plate Editor. Click this button to review the data for the selected plate and create images of the results in the Template view. When the batch review is complete, the rest of the buttons in the Review Control group become enabled. The image for the selected plot appears in the image view (Figure 2-21 on page 83).

⚠ **NOTICE** If you press the Generate Images button and the template view is not displayed or one or more plots are hidden (eg, the Plate Editor overlaps the plots), the following error message appears.



Click OK and then resize the Plate Editor so that all plots are visible.

Figure 2-21 Image generated



- *Plot Selector* drop-down menu—Click to select the generated image of the plot you want to view. The drop down menus lists all plots shown in the template view. Plots are identified by axes names. Plots display the gating defined in the template.

- *Play* button—Click to begin viewing generated images of plots for every well containing data. Use the Image Display Time control to adjust the speed at which images are displayed.

- *Stop* button—Becomes enabled after you click the Play button. Click the Stop button to stop displaying images. The last image displayed will remain on the image view.

- *Direction Arrow* buttons—Use the forward arrow (>) to go forward one image (well). Use the back arrow (<) to go backward one image (well).

- *Sequence Direction* button—The Sequence Direction button indicates the direction the images are being displayed. The button toggles between forward and backward. The forward arrows (>>>) indicate that images are moving from first to last. The backward arrows (<<<) indicate that images are moving from first to last. The default sequence is forward.

- *Position Indicator* display—Displays the number of the image being viewed. The first image begins at 0.

- *Display Time* slider—For adjusting the amount of time each image (well) is displayed. You can choose between 0 seconds through 2 seconds. If you choose 0 seconds, the images flash on and off the image view as quickly as possible.

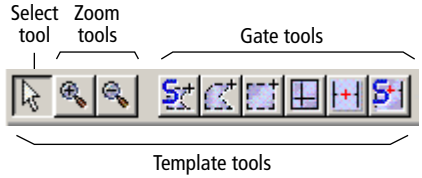
See Data Review on page 237 for a step-by-step procedure on reviewing data.

Plate View

To display the plate view, click the Plate tool ( ) in the application toolbar. The content of the plate view is determined by the sample layout defined in the Prepare tab of the Plate Editor. The wells in the plate view are colored according to whether wells contain a sample, whether wells have been selected for acquisition, and acquisition status. Wells are numbered according to acquisition order. Numbers in wells only appear if they are selected for acquisition, or are in the process of being acquired. The following table shows how the well appearance changes depending on status.

	A gray well indicates a well that does not contain a sample.
	A white well with a black border indicates a well that contains a sample.
	A green well with a black border indicates a well that is selected for acquisition.
	A green well with a yellow border indicates the well that is currently being acquired in Setup mode, but data is not being saved.
	A green well with an orange border indicates data is being saved for the well that is currently being acquired.
	A white well with a dark border and light green background in the plate view and a green well icon ( ) next to the well name in the Browser indicate that acquisition is complete and all events have been saved in an FCS file.

Template Toolbar



The following buttons are displayed in the template toolbar.

Select—selects (by clicking) objects in a template.

Zoom in—zooms in on a selected area.

Zoom out—zooms out to normal size.

Snap-To–Polygon Gate—draws a gate automatically around a distinct cluster on a dot plot after clicking on a population of interest. A Snap-To gate will automatically recalculate when new data is read into the plot or values are modified in the Snap-To region inspector.

Polygon Gate—creates a polygon gate around a population on a dot or contour plot.

Rectangle-Gate—creates a rectangular gate around a population on a dot plot.

Quadrant-Gate—divides a dot plot into four quadrant populations.

Auto-Interval Gate—draws an interval region (X direction only) automatically around a distinct population on a histogram plot. Once the region has been drawn, its shape and size remain constant, even when new data is read into the plot.

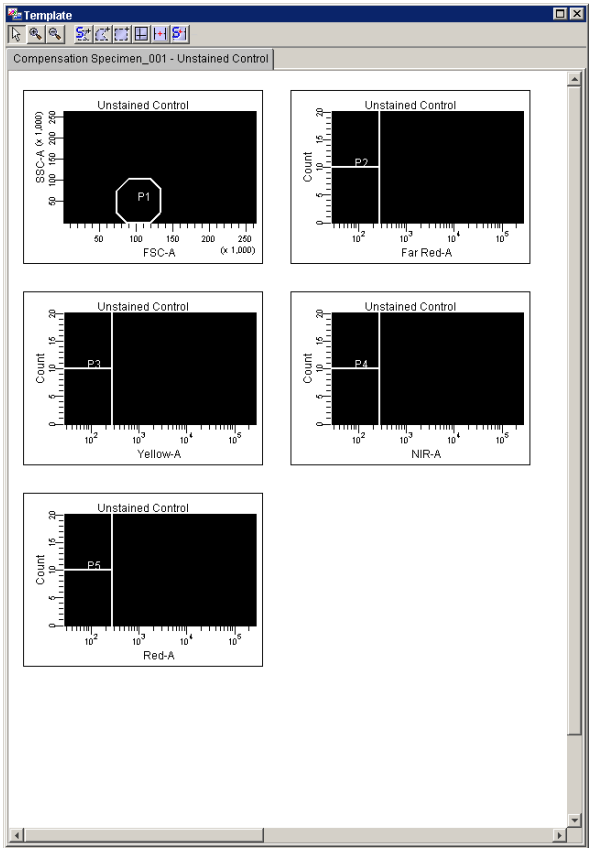
Snap-To–Interval Gate—draws an interval region (X direction only) automatically around a distinct cluster on a histogram or dot plot. Unlike an auto-interval region, a Snap-To–interval region will automatically recalculate when new data is read into the plot.

Template View

The Template view displays predefined plots and statistics for sample wells. You can create and adjust gates and display and customize statistics. Predefined plots and gates are displayed for each setup well when setup wells are defined. By customizing elements within the Template view (plot axes, gates, statistics, loader settings), you can create a template for each type of experiment you run to streamline acquisition and analysis of multiple sets of data.

To display the Template view (Figure 2-22), click the Template tool ( ) in the application toolbar.

Figure 2-22 Template view from 4-color template



Software Shutdown

Choose File > Quit to quit the software.

Any changes to an open experiment are automatically saved when you quit the application.

3

Instrument Overview

This chapter provides an overview of the BD FACSArray bioanalyzer and how it works, describes the components you will use to monitor and control the bioanalyzer, and contains a list of compatible multiwell plates.

The following topics are covered in this chapter:

- Theory of Operation on page 90
- Instrument Components on page 92
- Instrument and Acquisition Controls on page 97

Theory of Operation

The technology used by the BD FACSArray bioanalyzer optically measures the physical attributes of individual particles. These particles can be cells or beads. The major attributes measured are a particle's relative size, relative granularity or internal refractive index, and relative fluorescence intensity.

- Light scatter by particles is a complex process that is difficult to predict except for simple particles. However, for most BD FACSArray applications, some general principles apply.

  Forward scatter (FSC) depends on a particle's relative size, so in general, the larger the particle, the higher the FSC value. FSC also depends on the shape and contents of the particles. The FSC parameter in the BD FACSArray bioanalyzer can detect particles ranging from 2.5 µm to 50 µm in diameter.

  Side scatter (SSC) depends on a particle's relative size, and to a considerable degree, the internal granularity and content. For many cell types, particles with more granularity have a higher SSC value. The SSC parameter in the BD FACSArray bioanalyzer can discriminate more homogeneous cells and highly granular cell types.

  For particles greater than 5 µm in diameter (or length), the width of the FSC or SSC pulses (FSC-W or SSC-W) also measures of particle size because they measure the time that a particle takes to transit the laser beam. The larger the particle, the longer it takes to move through the laser beam. In some cases, the pulse width measurement is the most effective way to discriminate particles on the basis of size. An example of this is in discriminating two cells stuck together (a doublet) from a single large cell.

- Fluorescence intensity is detected as four different parameters covering the following spectral ranges.

  - Yellow (564–606 nm)
  - Red (653–669 nm)
  - Far Red (>685 nm)
  - Near Infra Red (NIR) (750–810 nm)

The BD FACSArray bioanalyzer uses this technology to analyze cells directly for cellular proteins and other constituents. Cell-associated proteins can be detected on the cell surface or intracellularly using up to four fluorescently labeled antibodies. Proteins that are secreted or present in cell lysates can be detected and quantified with the mutiplexed fluorescent BD cytometric bead array (BD CBA) technology.

How the BD FACSArray Bioanalyzer Works

First, samples are stained with fluorescent probes, such as fluorescently labeled antibody reagents, or they are prepared with BD CBA multiplex reagents. Stained samples, in a 96-well plate, are loaded into the BD FACSArray bioanalyzer.

The samples are mixed and then aspirated by the sample probe. The syringe pump injects the sample, carried in a sheath fluid stream, into the optical cuvette.

Particles in the sample stream intercept first a red laser beam and then a green laser beam. The particles are measured as they flow, one at a time, through the laser light in the optical cuvette. The laser light scatters off each particle as FSC and SSC. The fluorochromes or fluorescent dyes are energized by the lasers and emit fluorescent signals.

Signals given off by the particles are collected by optical detectors. The FSC signal is collected by the FSC photodiode. The SSC and fluorescent light are efficiently collected by a high numerical aperture lens that is perpendicular to the laser path and then focused into a series of optical detectors. The collected light is spectrally split by dichroic mirrors and limited to a fixed spectral range by a filter on each detector (SSC, Yellow, Far Red, Red, and NIR).

The optical signals are converted to proportional electronic signals (voltage pulses that are typically 5 µsecs in duration). The electronic signals are sampled ten times per µsec with analog-to-digital converters that measure 16,384 discrete intervals (14 “bits”). The total light emitted by a particle is measured as the area under a pulse (eg, Yellow-Area), and is obtained by summing all the sampled digital measurements (typically more than 50) of the pulse. The measured values of the pulses for each particle are sent to the computer for further processing and analysis. The data can then be analyzed by the BD FACSArray system software. CBA data is analyzed in BD CBA software.

Instrument Components

The BD FACSArray bioanalyzer consists of the following components. See Figure 3-1 on page 94 to view the location of each component.

- digital electronics—convert optical signals into proportional electronic signals that are then converted to digital values
- particulate filters
  - sheath filter—filters out particulates in the sheath fluid
  - air filters—prevent dust from entering the system and filter vented air
- lasers—provide the excitation source to energize fluorochromes conjugated to antibodies and fluorescent dyes and run on the bioanalyzer
  - green laser—emits 532 nm light. Fluorescence excited by this wavelength is detected in the Yellow and Far Red detectors.
  - red laser—emits 635 nm light. Fluorescence excited by this wavelength is detected in the Red and NIR detectors.
- optical cuvette—directs the sample stream containing cells or beads into single file where they intercept the laser beam
- optical detectors—collect scattered and emitted fluorescent light and convert the signals to equivalent electronic signals
  - PMTs—photomultiplier tubes (SSC, Yellow, Red)
  - APDs—avalanche photodiodes (Far Red, NIR)
  - PIN diode (FSC)
- optical filter—passes only light in a selected spectral range

- plate sampler—introduces the sample from the well and the multiwell plate into the bioanalyzer; houses the plate holder and sample probe

  - plate holder—holds the plate and moves it in the Y direction during loading and unloading of the plate sampler and during well acquisition

  - injection port—receives aspirated sample and delivers it to the optical cuvette; serves as probe wash station.

  - sample probe—aspirates sample while moving from well to well in the X and Z directions, and delivers sample to the injection port

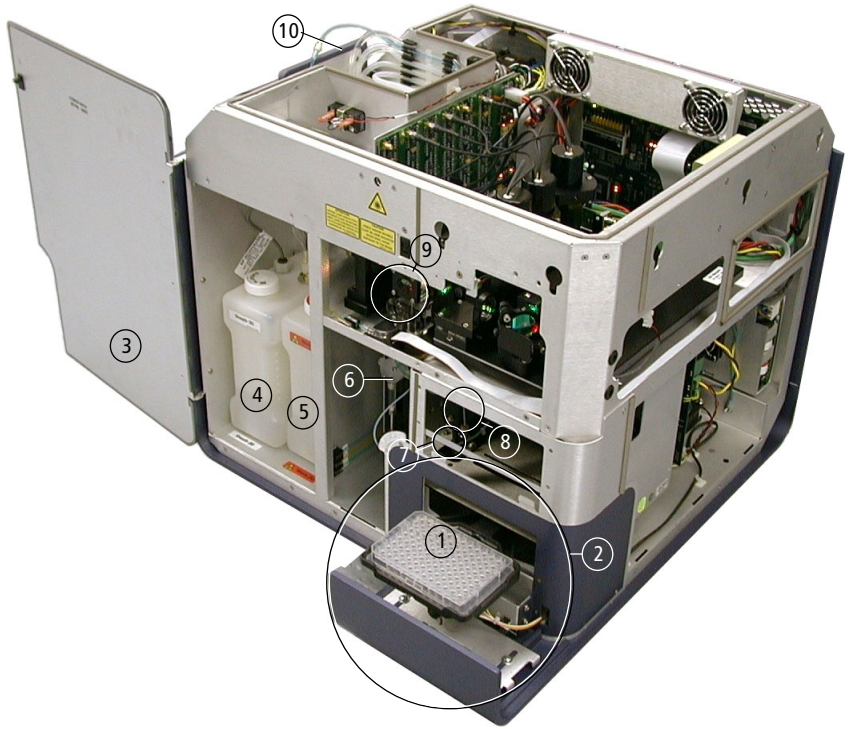
  - syringe pump—mixes the sample prior to sample acquisition by aspirating and expelling sample, through the sample probe, back into the well; aspirates and injects the sample into the optical cuvette; transports samples, after acquisition, to the waste tank; flushes the sample tubing with sheath fluid upon startup and shutdown.

- sheath tank (nonpressurized)—holds 4 L of BD FACS™ sheath solution with surfactant

⚠ **NOTICE** To ensure optimal reproducible results, use only BD FACS sheath solution with surfactant in the bioanalyzer and only fill the sheath tank up to the line marked on the tank.

- waste tank—holds 4 L of waste fluid

Figure 3-1 BD FACSArray bioanalyzer components



1	plate holder	6	syringe
2	plate sampler	7	injection port
3	front compartment door	8	sample probe
4	sheath tank	9	optical cuvette
5	waste tank	10	filter compartment

Bioanalyzer Configuration

The optical configuration of the BD FACSArray bioanalyzer is set and cannot be altered. Table 3-1 shows the instrument configuration along with the recommended fluorochromes to be used with the bioanalyzer for immunofluorescence.

Table 3-1 BD FACSArray Bioanalyzer Configuration

Parameter	Fluorochrome	Filter	Laser	Wavelength (nm)
FSC	NA	NA	Green	532
SSC	NA	NA	Green	532
Yellow	• PE[a]	585/42	Green	532
Far Red	• PE-Cy7[a] • PerCP Cy5.5[a]	685 LP	Green	532
Red	• APC[a] • Alexa Fluor® 647	661/16	Red	635
NIR[b]	• APC-Cy7[a]	780/60	Red	635

a. See page 2 for patent information.
b. NIR=Near Infrared

Optical Spillover

All fluorochromes are excitable by a range of wavelengths and emit a range of wavelengths. See Figure 3-2 for the emission spectra of each fluorochrome recommended for use with the BD FACSArray bioanalyzer.

Figure 3-2 Emission spectra of BD-recommended fluorochromes

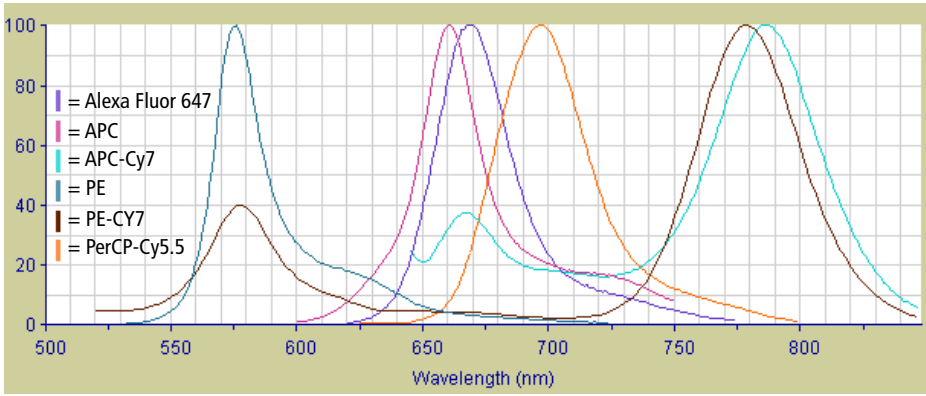


Figure 3-2 shows that some fluorochromes emit over a wide range of wavelengths into two or more different parameters, a primary parameter (showing the greatest signal) and secondary parameter(s) (showing lower signal). When using multiple fluorochromes to stain cellular samples, it is important to consider how much the fluorescence emission spectra of each dye overlaps the others.

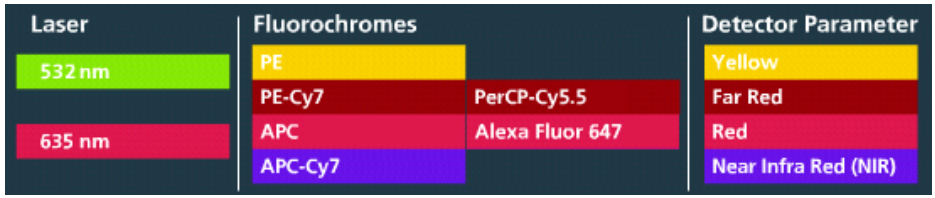
For example, Figure 3-2 shows that PE-Cy7 emits two ranges of wavelengths, one detected in the yellow (secondary) parameter and another detected in the far red (primary) parameter. Figure 3-2 shows that PE also emits wavelengths that are detected in the yellow parameter. So, the fluorescence emission spectra of both dyes are detected in the yellow parameter. This is referred to as optical spillover.

Ideally, only one color should be detected in each parameter. In this case, only the PE-Cy7 should be detected in the far red parameter and only the PE should be detected in the yellow parameter. You can choose to have BD FACSArray system software automatically calculate the amount of optical spillover and correct for the PE-Cy7 spillover in the yellow parameter. To do this, you need to add a setup well for each fluorochrome affected by optical spillover (PE-Cy7 and PE). See Four-Color Immunophenotyping Experiment on page 203.

When choosing fluorochromes to use in your experiment, BD recommends using combinations of fluorochromes with emission spectra that have minimal optical spillover, such as PE and APC or PE and Alexa Fluor 647. For this 2-color combination, you do not need to add setup samples to your experiment.

Figure 3-3 shows an example of fluorochromes excited by the green or red laser and the parameter in which they are detected.

Figure 3-3 Fluorochromes compatible for use with the BD FACSArray bioanalyzer



⚠ **NOTICE** It is necessary to add setup wells to your experiment only for cellular assays. Optical spillover correction is neither required nor calculated for CBA assays.

Instrument and Acquisition Controls

This section provides an overview of the components you will use to monitor and control the BD FACSArray bioanalyzer. See Chapter 2, Software Overview, on page 39, for information on other software elements.

You will use the following components to run and monitor the BD FACSArray bioanalyzer.

- Bioanalyzer components
  - Primary power switch
  - Secondary power button
  - LED indicator

- Software components
  - Acquire tab in Plate Editor
  - Acquisition Status frame
  - Inspector
  - Instrument frame
  - Wizard views

Instrument Controls

Primary Power Switch

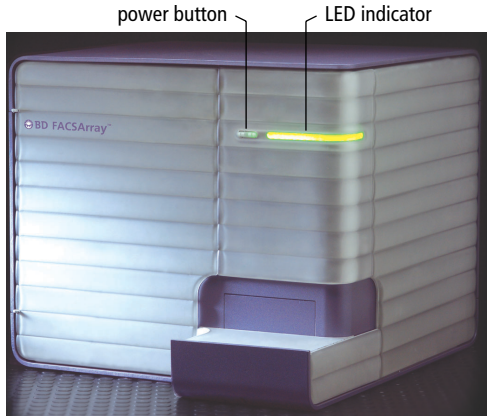
The bioanalyzer's primary power switch is located on the back of the instrument on the lower right side.

NOTICE When you shut off the bioanalyzer using the secondary power button, a relay shuts off power to all components of the bioanalyzer except the main power supply. The fan on this power supply remains on, so you will hear fan noise.

Secondary Power Button

The BD FACSArray secondary power button is located on the upper front panel of the instrument. See Figure 3-4 on page 99.

Figure 3-4 Location of power button and LED indicator on the BD FACSArray bioanalyzer



LED Indicator

The LED indicator, located on the upper front panel of the bioanalyzer, is illuminated when the power is on. The LED indicator will change color depending on instrument status. The following table shows LED indicator colors and their meaning.

LED Color	LED State	Instrument Status
Green	Unblinking	Instrument ready to acquire
Amber	Unblinking	Instrument not ready to acquire
Amber	Blinking	Fluidics tanks need attention
Red	Unblinking	System error; check Status tab in Instrument frame for status

See Troubleshooting on page 337 for more information.

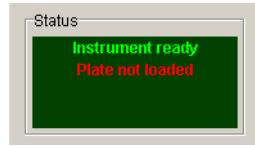
Software Controls

Acquire Tab in Plate Editor

The Acquire tab in the Plate Editor contains bioanalyzer controls. You can access these controls from any of the four Workspaces on the Acquire tab (see Figure 3-5 on page 101). See Acquire Tab on page 77 for more information about these controls.

- Status box

  The Status box displays the status of the instrument (for example, Instrument ready, Fluidics initializing, Instrument not connected, etc.) and whether a plate is loaded in the plate sampler.



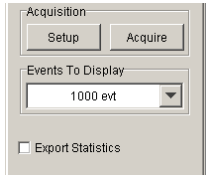
⚠ **NOTICE** To avoid damaging the bioanalyzer, do not quit the software while the fluidics are initializing.

- Load and Unload Plate buttons

  - The Load button is enabled when the Status box displays *Plate not loaded*.

  - The Unload button is enabled when events are not being acquired and the Status box displays *Plate loaded*.

- Setup and Acquire buttons

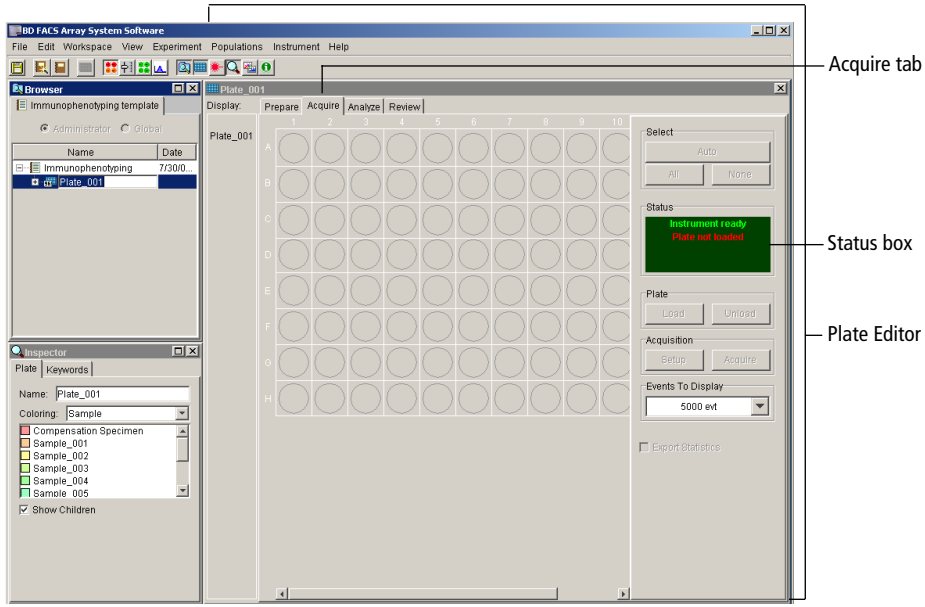


  - The Setup button is enabled when a plate has been loaded into the instrument and at least one well has been selected for acquisition.

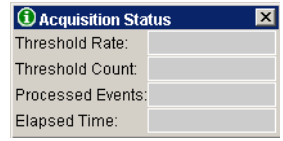
Clicking the Setup button begins sampling from the first well and events appear in the plots, although data is not being saved.

- The Acquire button is enabled when a plate has been loaded into the instrument and at least one well has been selected for acquisition. Clicking the Acquire button begins acquisition of the first well, events appear in the plots, and data is saved.

Figure 3-5 Prepare workspace showing the Acquire tab of the Plate Editor



Acquisition Status Frame

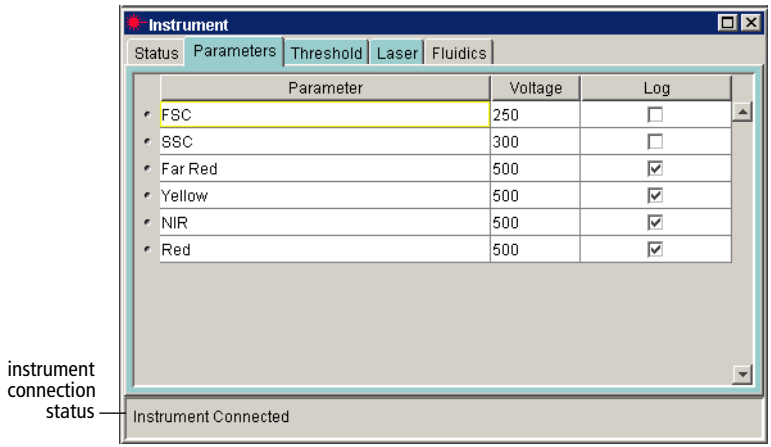


Use the Acquisition Status frame to display and monitor Threshold Rate, Threshold Count, Processed Events, and Elapsed Time during sample acquisition. Open the Acquisition Status frame by clicking the Acquisition Status tool ( ) in the application toolbar. You can also choose View > Acquisition Status. See Acquisition Status Frame on page 52 for more information.

Instrument Frame

The Instrument Frame is located in the Setup Workspace. However, you can view this frame in any workspace by clicking the Instrument tool ( ) in the application toolbar or choosing View > Instrument. The Instrument Frame contains five tabs: Status, Parameters, Threshold, Lasers, and Fluidics. You will use the Parameters tab to adjust instrument settings during sample optimization and setup. You will use the Threshold tab to set one or two threshold values to eliminate unwanted events, such as debris. The Status, Laser, and Fluidics tabs are View Only, and display any instrument errors, laser status, and fluidics pressure.

Figure 3-6 Instrument frame in the Setup workspace



Inspector

During experiment setup, you will use the Inspector (Figure 3-7 on page 103) to set loader settings prior to acquisition. Use the Experiment Inspector to view or change loader settings at the experiment level. Use the Well Inspector to view or change loader settings on a well-per-well basis. For more details on using the Inspector, see page 60. See Table 3-2 on page 103 for a description of each setting and range.

⚠ **NOTICE** With the exception of sample flow rate, sample volume, and mixing volume, BD recommends using the default loader settings provided when using a BD template to set up your experiment. Change loader settings only for specialty applications and validate the new settings prior to routine use.

Figure 3-7 Experiment Inspector (left) vs Well Inspector (right) displaying loader settings

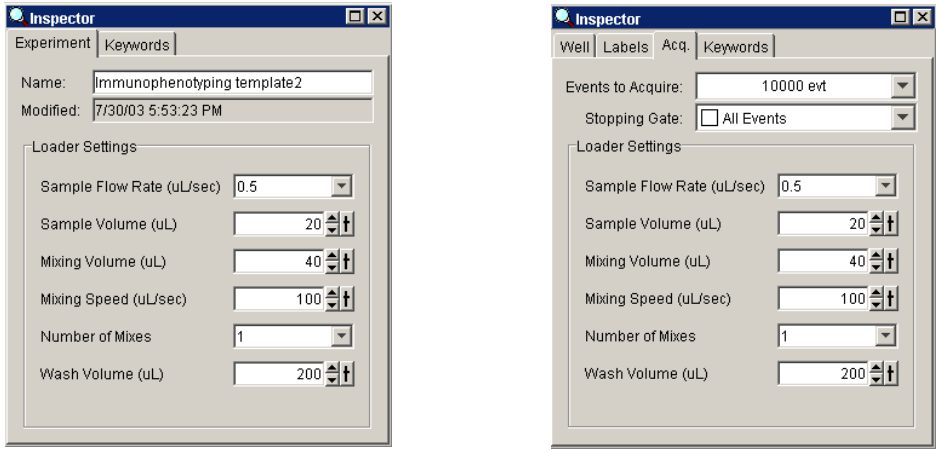


Table 3-2 Loader settings description and range of settings

Loader Setting	Range	Description
Sample Flow Rate (µL/sec)	0.5 to 3.0 µL/sec	Rate sample is injected into flow cell. Selectable in 0.5 µL/sec increments.
Sample Volume (µL)	5 to 100 µL	Volume of sample aspirate for analysis. Enter or select 5-100 µL. See page 104.
Mixing Volume (µL)	5 to 100 µL	Volume of sample aspirated and then dispensed back into well for mixing purposes. Enter or select 5-100 µL. See page 104.
Mixing Speed (µL/sec)	25 to 200 µL/sec	Rate that sample is dispensed into well to mix sample.

Loader Setting	Range	Description
Number of Mixes	0 to 10	Number of times sample is aspirated and dispensed back into well.
Wash Volume (µL)	200 to 800 µL	Volume of sheath fluid to wash sample probe inbetween wells.

Understanding Volumes

There are different volumes to take into consideration when choosing loader settings. Figure 3-8 shows different well volumes and Table 3-3 defines each volume type.

Figure 3-8 Well volumes

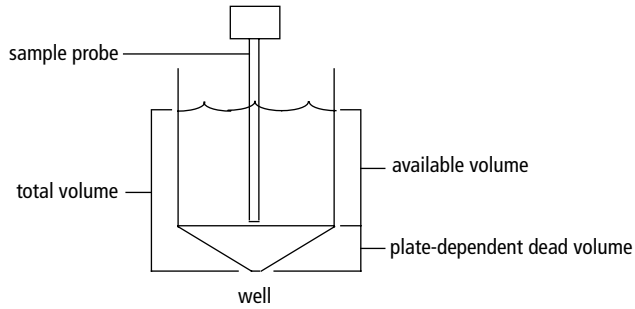


Table 3-3 Volume Type

Volume Type	Definition
Well volume	Volume that well can hold.
Total volume	= Volume pipetted into well – Aspirated volume(s)
Dead volume	Plate-dependent volume that sample probe cannot reach to aspirate. Refer to Plate Definition Guide.
Available volume	= Total volume – Dead volume
Sample volume	Volume aspirated for analysis.

Volume Type	Definition
Overhead volume	Additional volume (20 µL) aspirated in order to process sample.
Aspirated volume	= Sample volume + Overhead volume
Mixing volume	Volume half to three-quarters the available volume. **NOTICE** To ensure proper mixing, BD Biosciences recommends using a mix volume that is a half to three-quarters the available volume. **NOTICE** A mix volume that is larger than the available volume introduces air bubbles into the sample.

Experiment Wizard Views

You will use the views in the Experiment Wizard to set up an experiment. Experiment setup includes selecting the loader settings and instrument settings. For detailed information on setting up a cellular experiment using the Experiment Wizard, see Creating an Experiment Using the Wizard on page 204. For information on setting up an experiment for CBA analysis, see Creating a BD CBA Experiment Using the Wizard on page 169.

You can also set up an experiment manually. See Creating an Experiment Manually on page 238 for instructions.

4

Tools for Data Analysis

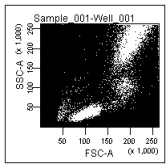
During analysis, the template is used to display acquired data in plots, and gates are used to define populations of interest. BD FACSArray system software analyzes the gated data and calculates statistics that you can print or export. This chapter provides an overview of data analysis.

The following topics are covered in this chapter:

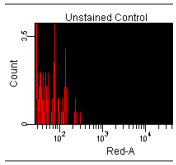
- Plots on page 108
- Gates on page 117
- Population Hierarchy on page 126
- Statistics on page 131
- Template Elements on page 138

Plots

Multiparameter data events can be displayed in dot plots or histograms.



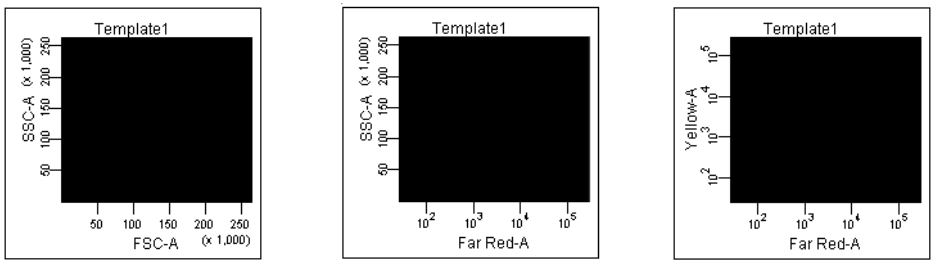
Dot Plot—graphical representation of two-parameter data, where each axis displays the signal intensity of one parameter and each dot represents one or more events (cells or particles)



Histogram—graphical representation of single-parameter data, where the horizontal axis represents the increasing signal intensity of the parameter, and the vertical axis represents the number of events (counts)

Plots are predefined in each BD template. The number and type of plot varies depending on which template you use.

Figure 4-1 Plots displayed in Linear (left), Log (right), and both (center)



linear dot plot

dot plot with one log parameter

dot plot with two log parameters

Plots can display data in Linear or Log scale, or both (Figure 4-1). The range for Linear and Log data is the same. All Log plots are shown with four decades that range from 26 to 262,143. The following rules apply to data shown on a plot.

- Values that fall below 26 are drawn on the left axis.
- Values that fall above 262,143 are drawn on the right axis.
- Regions that are drawn with the border on the left axis (26) include all events that are drawn on the left axis. For this example, all events in channels ≤26 are included in the gate.
- Regions that are drawn with the border on the right axis (262,143) include all events that are drawn on the right axis. For this example, all events in channels ≥262,143 are included in the gate.
- Statistics are calculated using the linear values of the events, even if the parameter is displayed in log scale.

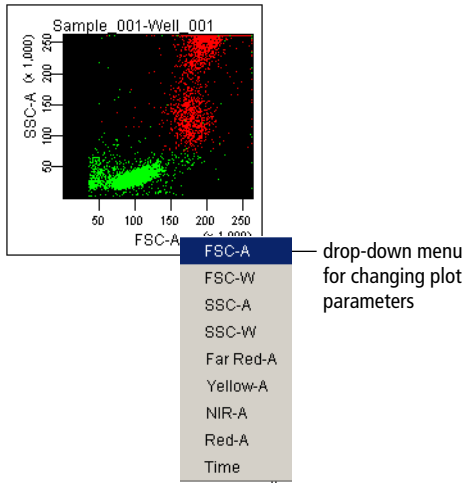
Editing Plots

Operations on plots are accessed through the Plot Inspector. For more information, see Using the Plot Inspector on page 112.

Changing Plot Parameters

Change plot parameters by clicking on the axis label in a plot and choosing a parameter from the drop-down menu that appears (Figure 4-2 on page 110). You can also change plot parameters using the Plot Inspector. All parameters specified in the Parameters tab of the Instrument frame are available. See Parameters Tab on page 67 for more information.

Figure 4-2 Changing plot parameters



Using the Zoom Tools

The Zoom-In ( ) and Zoom-Out ( ) tools are located in the Template toolbar.

- Use the Zoom-In tool to magnify an area of a plot to better view data.
- Use the Zoom-Out tool to return the plot to the default status.

Using the Zoom-In Tool

☑ **Tip** Using the Zoom-In tool to enlarge a small population allows you to set an accurate gate.

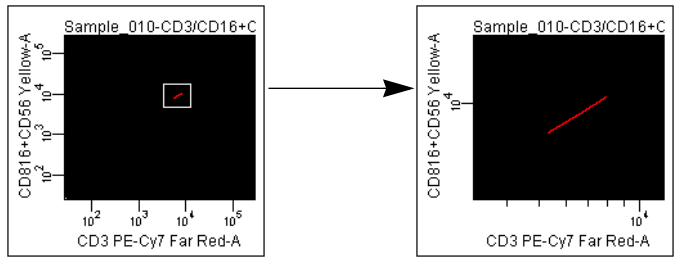
1 Select the Zoom-In tool and click in the plot to position the marker.

Click near the upper-left end of the population of interest.

2 Drag the mouse diagonally until the outline is the required size.

Release the mouse when the marker reaches the outer-right end of the population.

The selected area containing the population is magnified.



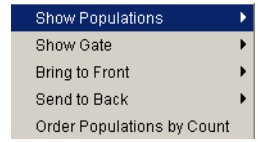
Selected area Magnified area

Using the Zoom-Out Tool

1. Select the Zoom-Out tool in the Template toolbar.
2. Click in the zoomed-in plot.

   The plot returns to the default scale.

Choosing Populations to Display



Right-click the upper border of any plot to access a contextual menu where you can choose from the following options.

- Show Populations—allows you to select which population(s) to display in the plot by selecting the population in the drop-down list. Choose multiple populations by reopening the menu. (Populations can also be selected in the Plot Inspector; see Formatting Plots on page 113.)

⚠ **NOTICE** If you try to gate on a population that has been assigned No Color (☒), no events appear in the plot.

- Show Gate—shows or hides a gate outline for selected populations in the plot. See Choosing Populations to Display on page 267. This option appears only after a gate has been created.

- Bring to Front—Draws the selected population in front of other populations in the plot. This is useful when the events of interest are obscured behind another population.

- Send to Back—Draws the selected population in back of other populations in the plot. This is useful for uncovering events of interest.

- Order Populations by Count—Draws the populations on the plot according to their count. The population with the fewest events is drawn on top of other populations in the plot.

Using the Plot Inspector

Use the Plot Inspector to format plots. When you select one or more plots in the Template view, different options are displayed in the Inspector.

☑ **Tip** Select multiple plots to make changes in the Inspector to all plots at once. Only options available to all selected plots are enabled.

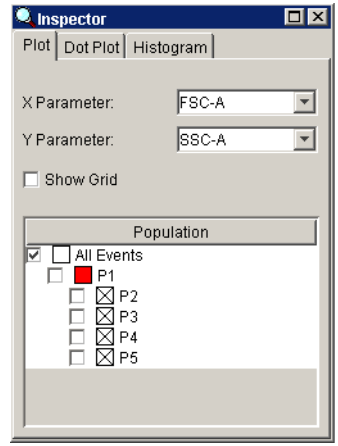
There are two components to the Plot Inspector, accessed by clicking the tabs at the top of the Inspector. The components in the tabs differ depending on whether you are editing a dot plot or a histogram.

Formatting Plots

Use the Plot tab of the Inspector to view the well associated with the plot, to view or change plot parameters, to specify the populations to show in the plot, and to show or hide a plot grid.

- Change the plot parameters by choosing a parameter from the X or Y Parameter drop-down menu (only the X parameter can be changed for histograms). All parameters specified in the Parameters tab (in the Instrument frame) are available. See Parameters Tab on page 67 for more information.

  NOTICE You can also change parameters on a plot by clicking the axis label and choosing a different parameter from the menu that appears. See Changing Plot Parameters on page 109.



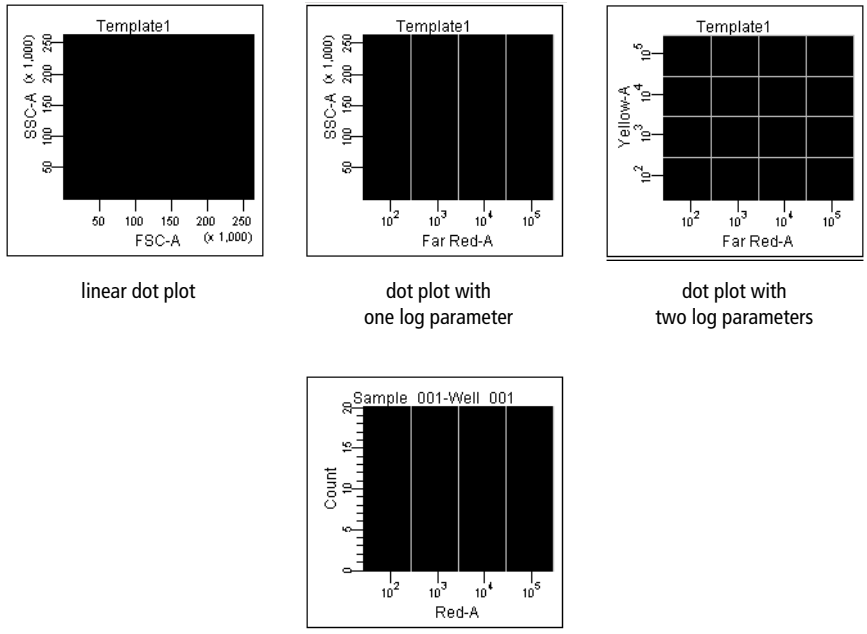
- Select the checkbox for each population to display in the plot. Alternatively, deselect a checkbox to hide the population.

  NOTICE You can also specify populations using the plot contextual menu. See Choosing Populations to Display on page 111.

- Select the Show Grid checkbox to display gridlines at each log decade in a log plot (Figure 4-3 on page 114).

  Gridlines are shown only if the Log checkbox is selected for the parameter shown in the plot. See the following examples.

Figure 4-3 Grid lines displayed in plots



linear dot plot

dot plot with one log parameter

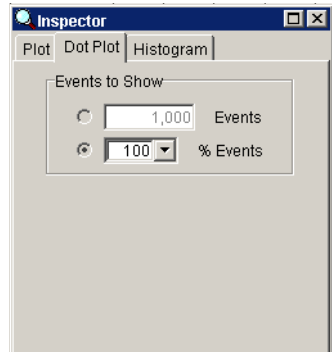
dot plot with two log parameters

histogram with log parameter

Formatting Dot Plots

Use the Dot Plot tab of the Inspector to specify how many events to show in the plot during analysis. Choose from the following options.

- Select the upper radio button and enter any number of events, from 1% of the total events to the total number of events acquired.
- Select the lower radio button and choose a percentage of the total events from the drop-down menu, or enter a percentage value in the field. The percentage is determined from the total number of events, eg, displaying 25% of events for a 4,000-event file will show every fourth event, not the first 1,000 events.

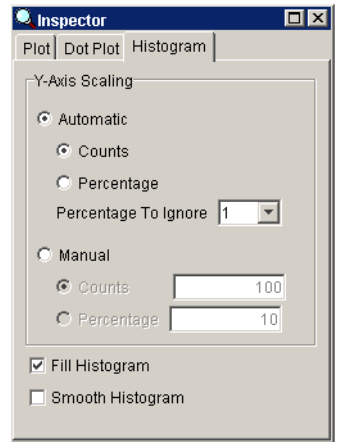


The minimum number of events to display is 1% of the total number of events.

NOTICE The number and percentage of total options are enabled only if the well for the selected plot contains data.

Formatting Histograms

Use the Histogram tab of the Inspector to format histograms. The y-axis scale can show either event counts or percentage of events in the histogram. For either method, you can set the maximum value or have it automatically calculated by the software.



- Select Automatic Counts if you want the y-axis to scale automatically to the highest peak in all the histograms.

- Select Automatic Percentage if you want the histogram to scale automatically to the highest percentage of the histogram data.

  The software finds the highest peak in each histogram and divides the number of events in the highest peak by the total number of events in the histogram. This percentage is used as the maximum value for the y-axis, and changes automatically as the data displayed in the plot changes.

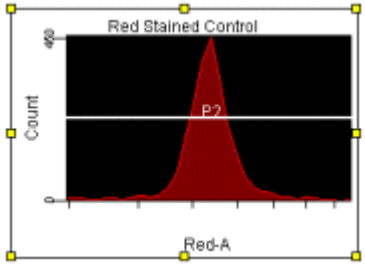
- Select a value from the Percentage to Ignore menu to disregard outlying events when calculating the y-axis scale.

  A high number of events at either end of the x-axis can skew the maximum value. When a value is specified, the software disregards the selected percentage of bins at each end of the x-axis when automatically calculating the y-axis scale.

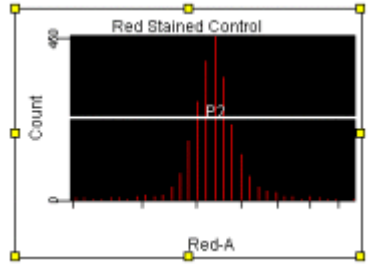
- Select Manual Counts to display a fixed count on the y-axis. Enter an integer between 1 and 50,000 events in the numeric field.

- Select Manual Percentage to display a fixed percentage value on the y-axis. Enter an integer between 1 and 100 in the numeric field.

- Select the Fill Histogram checkbox to fill in the area between histogram peaks. Deselecting the checkbox will show the individual bins. (This effect is apparent only on a zoomed-in histogram, where individual channel bins are visible.)

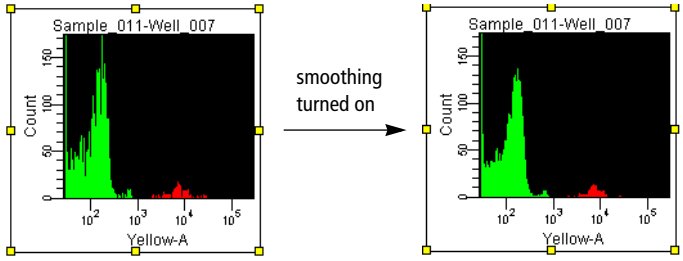


zoomed filled histogram



zoomed unfilled histogram

- Select the Smooth Histogram checkbox to smooth the histogram peaks.



unsmoothed unfilled histogram

unsmoothed filled histogram

Gates

Gates are used to identify subsets of data, or *populations*. Populations defined by gates can be used to generate statistics and limit the number of events collected or stored in the database. You can restrict a plot to display one or more populations. You can display populations in a hierarchical view and use the Population Hierarchy view to subset defined populations.

Gates are defined using tools in the Template toolbar or commands in the Populations menu. This section presents a description of the gating tools and the Population Hierarchy view. You can practice using the gating tools described in this section by performing the gating exercise in Chapter 7; see Advanced Gating Features on page 258.

Defining Gated Populations

Use the Gating tools ( ) in the Template toolbar to define gated populations by drawing in a plot or clicking on a population in a plot. After defining gated populations, you can combine them to create joined, intersected, or inverted gates; see Defining a Derived Gate on page 129.

☑ **Tip** Create multiple gates by holding down the Ctrl-key while selecting a Gate tool. The tool will remain selected until you press the Esc-key, select another tool, or click on the same tool again.

Creating Automatic Gates

The Snap-To Polygon and Snap-To Interval gates can be used to automate population analysis in plots.

Always inspect populations defined by automatic gates to ensure all required events have been included.

Snap-To Polygon Gates

Select the Snap-To Polygon gate tool ( ) and click on a distinct cluster in a plot.

A polygon gate is automatically drawn around the population after the data has been saved. Snap-To gates are automatically redrawn when the data in the gate changes.

NOTICE Snap-To gates do not work well with diffuse clusters or rare events.

☑ **Tip** Snap-To–Polygon gates are especially useful when performing batch analysis. A Snap-To Polygon gate will change under the following circumstances.

- after saving data, if the Snap-To gate was created during live acquisition or on a plot without any data displayed
- if a change to the gate hierarchy results in a change to the data appearing in the Snap-To gate

After moving a vertex, the Snap-To gate does not readjust automatically. You can force the gate to readjust by right-clicking on the gate boundary or on the population in the Population Hierarchy view (see Using the Population Hierarchy View on page 127) and choosing Recalculate from the contextual menu that appears.

Snap-To Interval Gates

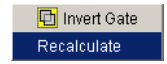
Select the Snap-To Interval gate tool ( ) and click on a distinct cluster in a histogram or dot plot.

An interval gate is automatically drawn that bounds the cluster in the X direction. A default interval gate is drawn on the plot if there are no events in the plot, no cluster can be found, or during setup mode.

☑ **Tip** Snap-To–Interval gates are especially useful when performing batch analysis.

A Snap-To Interval gate will change under the following circumstances.

- after saving data, if the Snap-To Interval gate was created during live acquisition or on a plot without any data displayed
- if a change to the gate hierarchy results in a change to the data appearing in the Snap-To Interval gate



After moving a boundary, the Snap-To Interval gate does not readjust automatically. You can force the gate to readjust by right-clicking on the gate boundary or on the population in the Population Hierarchy view (see Using the Population Hierarchy View on page 127) and choosing Recalculate from the contextual menu that appears.

Auto-Interval Gates

Select the Auto-Interval Gate tool ( ) and click on a distinct cluster in a histogram or dot plot. An interval gate is automatically drawn that bounds the cluster in the X direction.

When using Auto-Interval gates, keep in mind the following limitations.

- Auto-Interval gates can be created only in plots that contain a distinct population cluster.
- Auto-Interval gates remain fixed after they are drawn.
- Auto-Interval gates are not recommended for use during data acquisition because the gate is created immediately after you click in the plot.

Creating Gates with Other Gating Tools

You can draw a Polygon, Rectangle, Quadrant, or Interval gate using the appropriate tool in the Template toolbar. After a population is defined, it is assigned a color and position in the Population Hierarchy view (see Population Hierarchy on page 126).

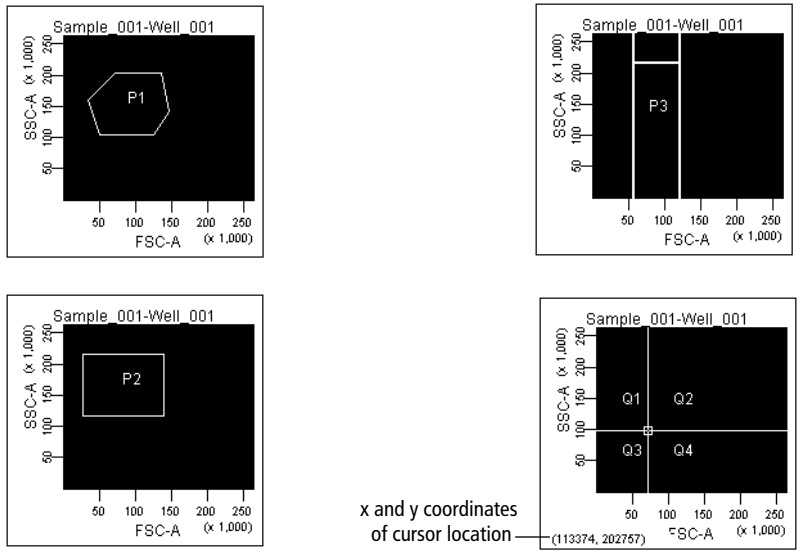
Populations defined by intervals and quadrants do not have a color assigned. You can change the color of a population using the Population Hierarchy Inspector; see Changing the Color of Events on page 128.

	Use the Polygon-gate tool to create a Polygon gate on a dot plot. **1** Select the tool and click in the plot to establish the starting point (first vertex). **2** Move the cursor to create the next vertex and click. **3** Continue moving the cursor and setting vertices; double-click the last vertex to complete the gate.
	Use the Rectangle-gate tool to create a Rectangular gate on a dot plot. **1** Select the tool and click in the plot to position the gate. **2** Drag diagonally until the gate outline is the required size.
	Use the Quadrant-gate tool to divide a dot plot into four separate populations. Each quadrant population can be named and colored individually. Quadrant populations can be used for subsetting. **1** Select the tool and click in the plot. **2** Drag the mouse to position the intersection of the quadrant markers. The coordinates of the intersection appear at the bottom of the plot (Figure 4-4).

You can create up to 64 child gates per parent gate.

Examples of each type of gate are shown in Figure 4-4 on page 121. This figure illustrates how population colors defined by polygon and rectangular gates do not change after drawing an Interval or Quadrant gate.

Figure 4-4 Polygon (P1), Rectangle (P2), Interval (P3), and Quadrant (Q1–Q4) gates



Editing Gates

Any gate can be moved or resized, but you cannot add a vertex to or delete a vertex from an existing gate. Statistics are automatically updated after a gate is edited. Changes to a parent gate will affect all populations derived from that gate (see Population Hierarchy on page 126).

1 Click once on a gate to select it.

Selection handles will appear on the gate.

2 Make changes to the selected gate; click outside the plot to deselect it.

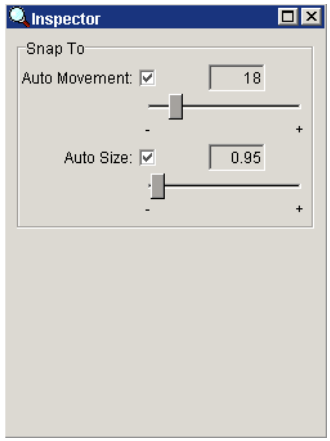
- To move a gate, drag the border of the gate. Note that the label moves with it. You can move just the gate label by dragging only the label.
- To resize a gate, drag any of the selection handles to a new location.
- To delete a gate, select the gate and choose Edit > Delete.

⚠ **NOTICE** To avoid confusion, keep gate labels close to the population they identify. Do not move quadrant labels out of their respective quadrants.

Editing Snap-To Gates

If you move one of the Snap-To gate vertices, the change will not be applied to subsequent wells. To make the change in all wells, use the Auto Size and Auto Movement fields in the Snap-To gate Inspector to change the Snap-To gate.

To adjust the movement and sizing of a Snap-To gate that applies to all wells in the plate, use the Auto Movement and Auto Size controls in the Snap-To Gate Inspector.



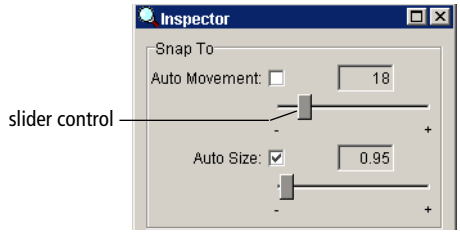
Adjusting the Movement of Snap-To Gates

When updating, a Snap-To gate searches for a cluster closest to the area it was originally placed. You can change how far the gate moves to find a new cluster by adjusting the Auto Movement value.

1 Click on a Snap-To gate inside a plot containing distinct populations.

2 In the Snap-To Gate Inspector, deselect the Auto Movement checkbox.

By default, the Auto Movement checkbox is selected. This limits movement of the Snap-To gate to 18 (not much movement).



3 Adjust the slider control toward the right until the gate encompasses the cluster of interest.

Alternatively, enter a value in the Auto Movement field and press Return on your computer keyboard.

The Auto Movement range is a percentage of the plot width, or resolution. A higher Auto Movement value allows the Snap-To gate to travel greater distances to locate a cluster. The Snap-To gate retains this setting for subsequent wells.

Adjusting the Size of Snap-To Gates

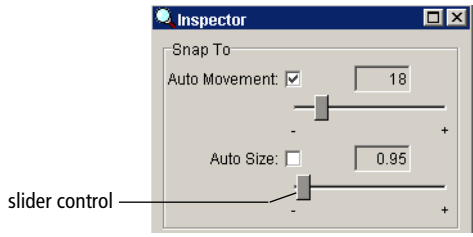
Cluster variability can cause the software to draw a Snap-To gate around only a portion of a cluster. Use the Auto Size feature to adjust automatic sizing of the Snap-To gate.

1 Click on a Snap-To gate inside a plot containing indistinct populations.

2 In the Snap-To Gate Inspector, deselect the Auto Size checkbox (Figure 4-5 on page 124).

The Auto Size checkbox is selected by default. This allows the software to automatically determine cluster size.

Figure 4-5 Snap-To Gate Inspector



3 Adjust the slider control toward the right until the gate encircles the entire cluster.

Alternatively, enter a value in the Auto Size field and press Return.

A higher Auto Size value allows the Snap-To gate to encompass a greater number of outlying events; a lower value restricts the gate to fewer outlying events. The Snap-To gate retains this setting for subsequent wells.

Tethering Snap-To Gates

A Snap-To gate requires a minimum number of events in order to find a cluster. When you want to automatically gate a small number of events or analyze an area of a plot that might or might not contain events, you can *tether* one or more manual gates to a Snap-To gate. Tethered gate(s) move relative to the Snap-To gate.

This feature is useful when you expect changes in the population of interest in relation to another population. You can use tethered gates to help automate rare event analysis. Tethered gates have the same properties as regular gates. For example, a plot can be gated and statistics can be generated from a tethered gate.

The following restrictions apply to tethered gates.

- Only gates with the same parameters as the Snap-To gate can be tethered.
- Only manually drawn gates can be tethered to Snap-To gates.
- Only one manual gate can be tethered to one Snap-To gate; however, one Snap-To gate can be tethered to many manually drawn gates.

- If you move, resize, or reshape the Snap-To gate, the tethered gate(s) remain the same. The Snap-To gate reverts to its previous position, size, or shape for subsequent wells.

- If you move the tethered gate, the relative position is remembered and used for subsequent wells.

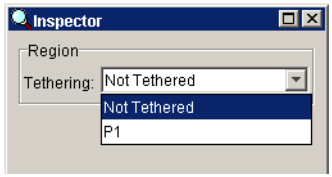
Follow these steps to create a tethered gate.

1 Create a Snap-To gate (P1) and a polygon gate (P2) in an appropriate plot.

2 Select P2 in the plot.

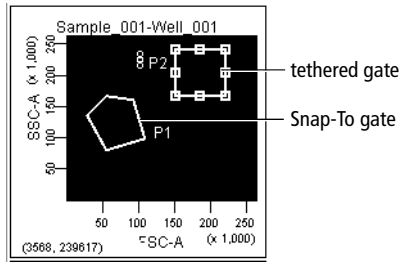
3 In the Gate Inspector, click the Tethering drop-down menu.

The drop-down menu lists all gates in the plot; only Snap-To gates are enabled. Snap-To gates on other plots with the same parameters also appear in the list.



4 Select P1 from the drop-down menu.

The border of the P2 gate changes and a chain-link icon appears next to the gate label to show that the gate is tethered. (A tethered gate has a bold outline similar to a Snap-To gate.)



Editing Gates During Acquisition

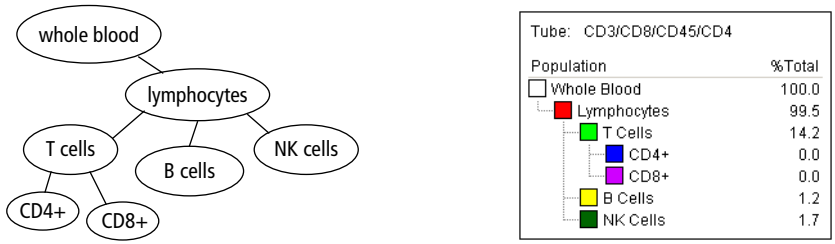
Gates can be edited during acquisition. Modifying a gate will change the classification of events. When a gate changes during acquisition, the plots will not clear. New data will be added to the plots, and all data that was saved before the change occurred will not be reprocessed until acquisition is complete.

Population Hierarchy

All defined gates are listed in a Population Hierarchy view. Use the Population Hierarchy to view defined populations and to display the relationship between different gated populations for a particular well.

For example, to subset a whole blood sample for immunophenotyping, you first identify the lymphocytes, and then individual cell populations. The Population Hierarchy view shows how these populations are identified by first subsetting lymphocytes from the whole blood sample (All Events), and then separating the lymphocytes into T, B, and NK cells.

Figure 4-6 Immunophenotyping hierarchy and corresponding Population Hierarchy view



☑ **Tip** If you want to create lots of gates or identify gates using large names, use the Multicolor template. The Multicolor template displays nine plots and the Population Hierarchy view is positioned to accommodate up to 20 gates without obstructing other views. The position and size of the Population Hierarchy view is fixed.

Using the Population Hierarchy View

Use the Population Hierarchy view to

- rename populations—Select any population and enter text to change its name. The new name will appear on all plots displaying that population.
- subset populations of events—See Advanced Gating Features on page 258.
- change the color of defined populations—Double-click on the color box next to the population name and choose a new color from the menu. See Changing the Color of Events on page 128.
- view the relative percentages of different populations—To see how these numbers are calculated, see Calculating Statistics on page 135.

  Because some events reside in more than one population, sibling percentages do not always add up to 100%.
- delete populations—Select any population and press the Delete key.

Using the Hierarchy, Population, and Gate Inspectors

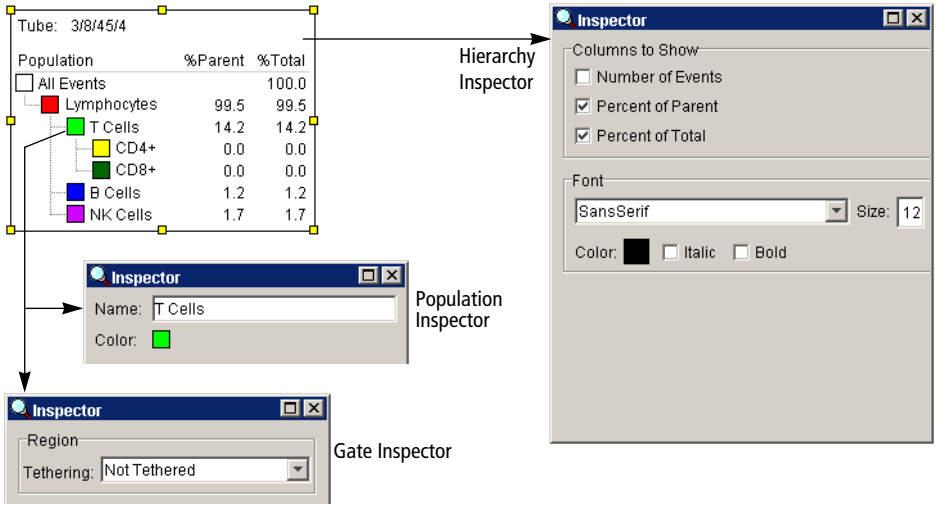
Do the following to view the different Inspectors.

- Click anywhere inside the population hierarchy and then click the Inspector tool in the application toolbar to see the Hierarchy Inspector.

  Use the Hierarchy Inspector to select which information should appear in the Hierarchy view and to change the font.
- Click on any population in the population hierarchy and then click the Inspector tool in the application toolbar to see the Population Inspector.

  Use the Population Inspector to change the name and color of a population.
- Click on any gate in the plot and then click the Inspector tool in the application toolbar to see the Region Inspector.

Use the Gate Inspector to change tethering properties of the gate and to delete gates from plots. See Tethering Snap-To Gates on page 124.



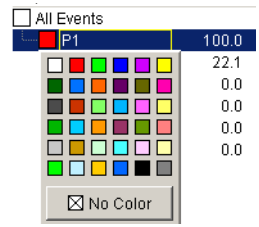
Changing the Color of Events

By default, populations defined by quadrants and intervals are not assigned a color (☒) in the Population Hierarchy view; they retain the color of their parent gate. For all other gate types, populations are assigned the color of the last gate they satisfy.

Population colors can be changed manually after a gate is defined. To change a population color, do the following.

1. Double-click on the color box in a Population Hierarchy view or click on the Color box in the Gate Inspector (shown here).

2. Choose a new color from the menu that appears or click the No Color box to leave the gated events uncolored.



⚠ **CAUTION** If you display a population that has been assigned No Color in a subsequent plot, no events will appear in the plot. If you plan to further subset any population, first assign it a color.

Creating Population Subsets

To restrict a subset to a certain population of events, do the following:

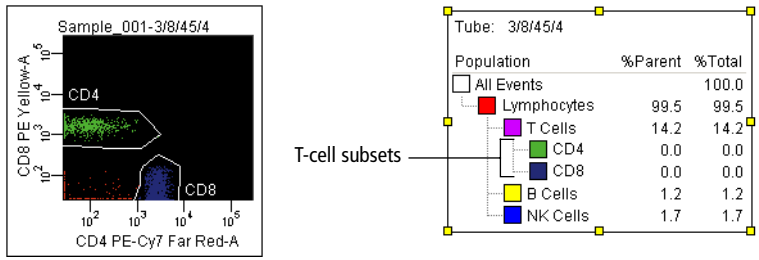
1. Select a population in the Population Hierarchy view.

   For example, click Lymphocytes.

2. Select the appropriate gating tool.

3. Draw a gate in a plot that shows all events.

   The new population appears indented below the selected population in the Population Hierarchy view.



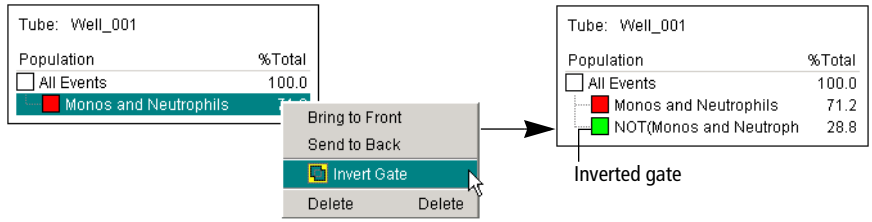
Defining a Derived Gate

You can use one or more previously defined gates to create a *derived gate*. There are two kinds of derived gates:

- Inverted gates use the NOT operator to select events outside a defined gate. Any event outside the specified gate satisfies the inverted gate.

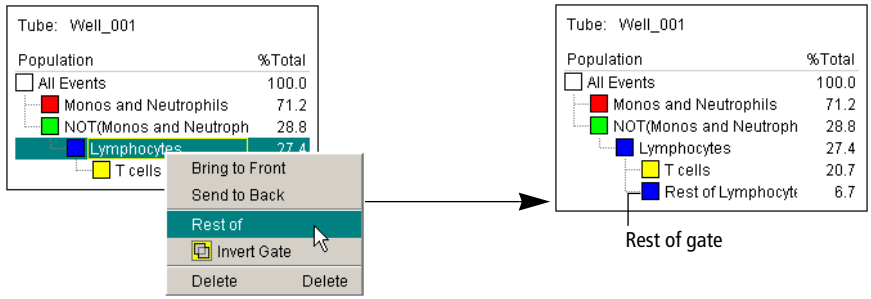
  To define an Inverted gate, right-click an existing population in the Population Hierarchy view and choose Invert Gate (Figure 4-7 on page 130).

Figure 4-7 Creating an Inverted gate



- *Rest of* gates select all remaining events that do not fall into any of the child gates of a parent gate. Thus, you can only access the *Rest of* option when you select a gate that already contains children.

  To define a *Rest of* gate, right-click a parent population in the Population Hierarchy view and choose *Rest of*. Events in the *Rest of* population retain the coloring of their parent unless you choose to change the color. The color was changed in the following example to illustrate the events.



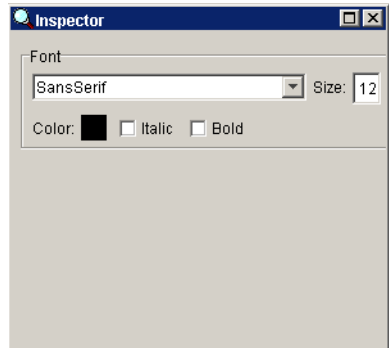
Statistics

BD FACSArray system software generates statistics from the linear values of acquired events. Statistics can be displayed for any parameter and calculated for any defined population. Statistics are displayed in a predefined Statistics view on the template, like a plot, and can be exported to a file.

Selecting Statistics to Display

Use the Statistics view editor to specify the statistics to display; use the Statistics Inspector to change the font of the statistics view. To access the Statistics View editor, do one of the following:

- Click the Edit Statistics View button in the Statistics Inspector.
- Right-click the Statistics view and choose Edit Statistics View from the contextual menu.

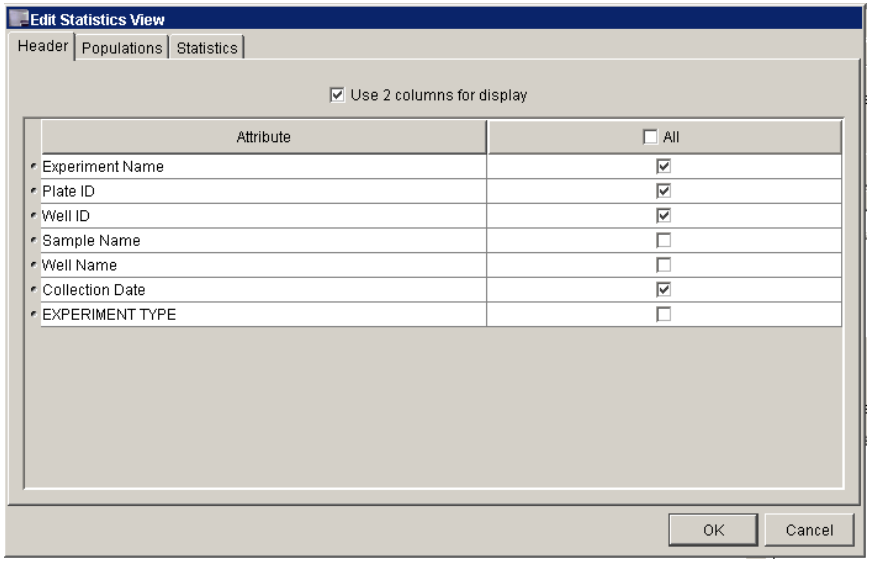


There are three components to the Statistics View editor, accessed by clicking the tabs labeled Header, Populations, and Statistics. See Figure 4-8 on page 132.

Editing Header Information

Use the Header tab to specify the information to include in the header of the statistics view.

Figure 4-8 Statistics View editor

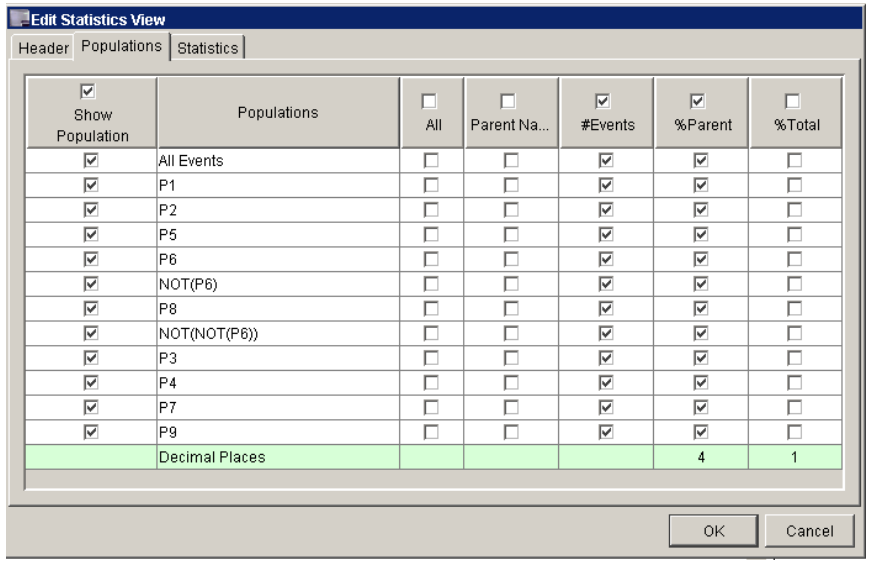


- Select the *Use 2 columns for display* checkbox to display header information in two columns.
- Display a header item by selecting its checkbox; delete an item from the header by deselecting its checkbox.
- Select the *All* checkbox to display all header items.

Experiment Name, Plate ID, Well ID, Well Name, and Collection Date are selected by default.

Editing Population Statistics

Use the Populations tab to define the populations listed in each row of the statistics view. The Populations table allows you to specify the populations and types of population statistics to be displayed.



To include a population in the statistics view, select the Show Population checkbox next to the population name. To include all populations, select the checkbox at the top of the column.

Specify additional population information to display by selecting the appropriate checkboxes.

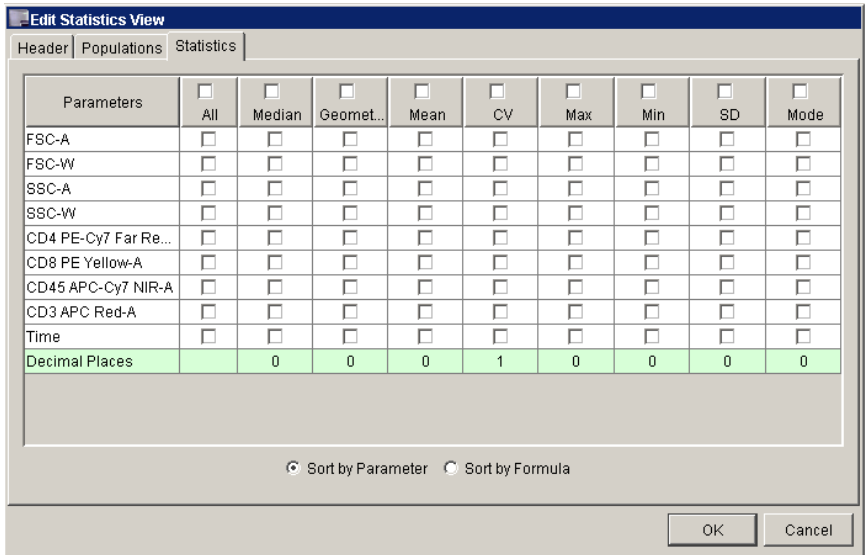
- Select the All checkbox next to a population name to display all information for that population.
- Select the checkbox in a column header to display that information for all selected populations.

Enter the number of integers (0 through 4) to display to the right of the decimal point for the %Parent and %Total Population statistics.

Delete a population or statistic from the statistics view by deselecting its checkbox.

Editing Parameter Statistics

Use the Statistics tab to specify which parameter statistics to be calculated and displayed in each column of the statistics view.



- Within each row, select checkboxes for each statistic to display for that parameter, or select the All checkbox to display all statistics for that parameter.
- Within each column, select the checkbox in a column header to display that statistic for all parameters.
- In the All column, select the checkbox above the column header to display all statistics for all parameters.
- In the Decimal Places row, enter the number of integers (0 through 4) to display to the right of the decimal point for the statistic in the column header.

- At the bottom of the editor, select a radio button to specify how statistics should be sorted: by Parameter (eg, PE Yellow Mean, PE Yellow CV, APC Red Mean, APC Red CV) or by Formula (eg, PE Yellow Mean, APC Red Mean, PE Yellow CV, APC Red CV).

  NOTICE Statistics can be displayed for any well parameter, including the Time parameter. If a label has been specified for a parameter, it will appear before the parameter name.

  For an explanation of how statistics are calculated, see the following section.

Calculating Statistics

All data originating from the digital electronics is linear and statistics are always calculated on linear data. When optical spillover has been applied, statistics are calculated on the optical spillover data.

Most statistics are shown in the statistics view, but certain statistics are shown only in the Population Hierarchy view. The following types of statistics can be calculated:

- Number of events—total number of events in the defined population
- Parent—name of the next population up in the hierarchy
- % Parent—number of events in the defined population divided by the number of events in the parent gate (next population up in the hierarchy), expressed as a percentage
- % Total—number of events in the defined population divided by the total number of events in the Tube (all events), expressed as a percentage
- Mean—average linear value for events in the defined population, defined as

$$\bar{X} = \left(\sum_{i=1}^{n} X_i\right) / n$$

where n = number of events in the population, and X_i is a value for a particular parameter, where i = 1 to n

- Geometric mean—logarithmic average of the events in the defined population

  This mean is less sensitive to outliers than the regular mean. The geometric mean is defined as

  $$\bar{X}geo = 10^{\left(\sum_{i=1}^{n} \log X_i\right)/n}$$

  where n = number of events in the population, and X_i is a value for a particular parameter, where i = 1 to n

- Standard deviation (SD)—a measure of the spread around the mean for events within a defined population, defined as

  $$SD = \sqrt{\sum_{i=1}^{n} (X_i - \bar{X})^2/(n-1)}$$

- Coefficient of variation (CV)—the SD divided by the mean within a defined population, expressed as a percentage

  The CV is defined as

  $$\text{Percent CV} = (SD/\bar{X}) \times 100$$

- Median—linear value with an equal number of values above and below it

  If the median of the data occurs between two values, those two values are added and divided by two to get the median.

  NOTICE During acquisition, BD FACSArray system software uses a faster but less accurate method to calculate the median. Thus, after analysis, the median value can differ slightly from what is observed during acquisition.

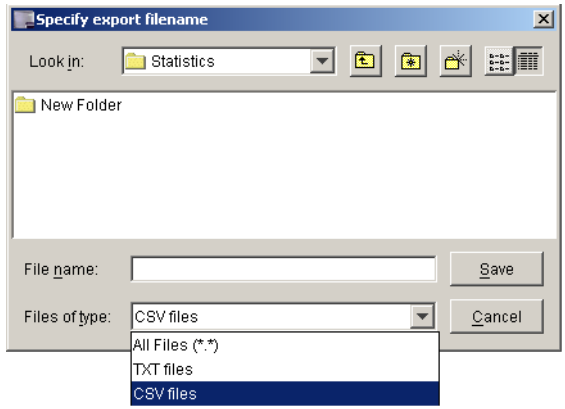
Exporting Statistics

You can export statistics from a selected statistics view for use in a spreadsheet, word processing, or other third-party application. Statistics information (including header text) is exported to a specified type of file. To export statistics, do the following.

1. Select the statistics view.

2. Choose File > Export > Statistics.

3. Enter a name for the statistics file and specify a storage location in the dialog box that appears.



4. Specify the file type (CSV or Text), and click Save.

   - Use CSV files (comma separated values) for spreadsheet applications such as Microsoft Excel.

     Any commas in the text of exported fields will cause the text to be split into two cells in the spreadsheet application.

   - Use TXT files for wordprocessing applications such as Microsoft Word.

Template Elements

Copying Template Elements

You can copy an image of the Template frame—including the plots and statistics—and resize it or edit it in a wordprocessing or graphics application.

1. Copy the Template to the clipboard.

   - To copy an image of the currently open workspace, press Alt-Print Screen.

   - To copy an image of the entire screen, press Print Screen.

2. Paste the image into an open window in the wordprocessing or graphics application.

   The image is stored in memory until it is pasted into another application.

NOTICE You can also print any of the workspace views using the above instructions.

Printing Templates

Choose one of the following commands from the File menu to set up for printing or to print Templates.

- Choose Page Setup to set the size of the printed page (ie, A4 vs letter), the orientation (portrait or landscape), and the margin size. Your options will vary depending on the printer configured with your workstation.

- Choose Print Preview to view a thumbnail of all printable pages at 10, 30, 50, or 100%. Click the close box to return to the template.

⚠ **NOTICE** Print Preview is a memory-intensive operation. When system memory is low, choosing the Print Preview command can cause the application to crash.

- Choose Print to print the active Template.

Objects in the Template frame might temporarily disappear during print spooling. The amount of time the Template appears blank depends on the size of the print file. Do not close the experiment or quit the application when this happens. The Template objects will immediately reappear when spooling is complete.

NOTICE Batch printing might take several minutes to complete.

Exporting Template Elements

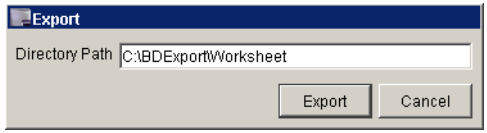
You can export selected elements from the Template view for use in a spreadsheet, word processing, or other third-party application. Statistics information (including header text) is exported to a specified type of file. To export Template elements, do the following.

1. Select the element(s) in the Template view.

   Choose Edit > Select All to select all elements in the Template view.

2. Choose File > Export > Template Elements.

3. Enter a name for the export file and specify a storage location in the dialog box that appears.



5

System Startup and Shutdown

The following topics are covered in this chapter:

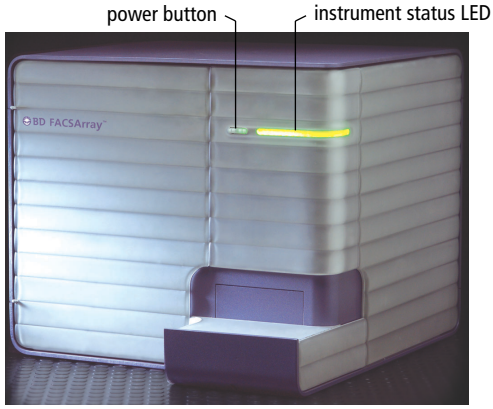
- Starting Up the BD FACSArray Bioanalyzer on page 142
- Performing Daily Inspection on page 145
- Starting Up the Workstation and Software on page 146
- Performing Instrument Quality Control on page 147
- Shutting Down the Instrument on page 166

Starting Up the BD FACSArray Bioanalyzer

WARNING All biological specimens and materials coming into contact with them can transmit potentially fatal disease. To prevent exposure to biohazardous agents, handle specimens and materials as if capable of transmitting infection. Dispose of waste using proper precautions and in accordance with local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

1. Press the power button to start up the BD FACSArray bioanalyzer.

   The button is located on the upper front panel of the bioanalyzer. The LED indicator is illuminated when the power is on.



2. Open the front compartment door of the bioanalyzer.

   Press, and then release the indented area in the middle portion of the front compartment door to open it.

3. Locate the sheath and waste tanks (Figure 5-1 on page 143).

Figure 5-1 Front compartment door open showing sheath and waste tanks

4 Check the fluid level in the sheath tank and fill to the line marked on the label with BD FACS sheath solution with surfactant, if necessary (Figure 5-2 on page 144).

⚠ **NOTICE** Use only BD FACS sheath solution with surfactant in the sheath tank of the BD FACSArray bioanalyzer. Using other types of sheath fluid might compromise instrument performance.

☣ **WARNING** To avoid overflow of potentially infected fluid from the waste tank, empty the waste tank if you add fluid to the sheath tank and keep the fluid level in the sheath tank below the mark on the tank label.

⚠ **NOTICE** To avoid leaking sheath fluid from the fluidic lines, do not service the sheath tank while in Setup or Acquire mode.

- Disconnect the sheath tank sensor jack (①) from the bioanalyzer.

  Press the metal tab in on the bioanalyzer fitting to release the connector.

- Disconnect the white connector (②) from the sheath tank.

  Press the metal tab in on the tank fitting to release the connector.

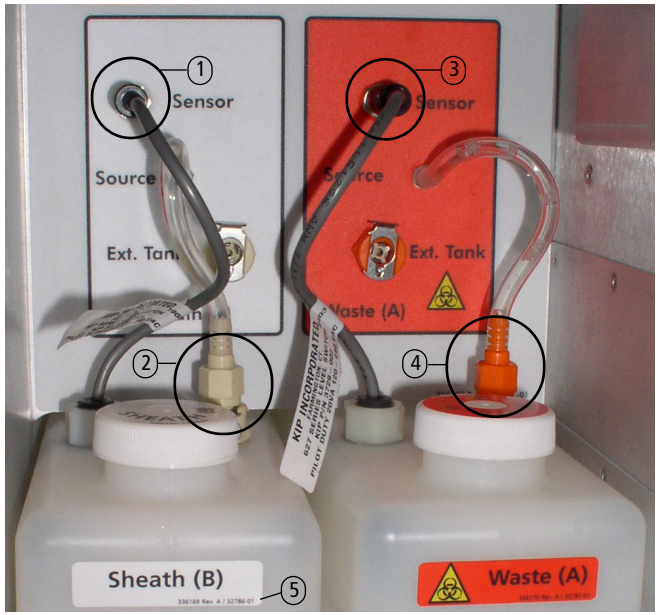
- Fill the sheath tank with BD FACS sheath solution with surfactant, being careful to keep the fluid level in the sheath tank below the mark on the tank label.

- Replace the sheath tank and connect the sheath tank sensor jack (①) to the green-ringed connector on the bioanalyzer.

  The sheath tank sensor jack is labeled *Sensor* on the Sheath (B) label on the back wall of the bioanalyzer.

- Plug the white connector (②) into the sheath tank.

Figure 5-2 Fluidics compartment



① sheath tank sensor
② sheath connector
③ waste tank sensor
④ waste connector
⑤ sheath tank fluid line

5 Check the fluid level in the waste tank and empty if full or if you added fluid to the sheath tank (Figure 5-2).

WARNING To avoid leaking potentially biohazardous waste from the fluidic lines, service the waste tank while the bioanalyzer is in standby mode only. Do not attempt to service the tank while in Setup or Acquire mode.

WARNING To avoid overflow of potentially infectious fluid, always empty the waste tank if you add fluid to the sheath tank and do not fill the sheath tank above the level marked on the sheath tank label.

- Disconnect the waste tank sensor jack (③) from the bioanalyzer.

  Press the metal tab in on the bioanalyzer fitting to release the connector.

- Disconnect the orange connector (④) from the waste tank.

  Press the metal tab in on the tank fitting to release the connector.

- Dispose of waste according to local regulations.

- Add 400 mL of undiluted bleach to the waste tank.

- Replace the waste tank and connect the waste tank sensor jack (③) to the red-ringed connector on the bioanalyzer.

  The waste tank sensor jack is labeled *Sensor* on the Waste (A) label on the back wall of the bioanalyzer.

- Plug the orange connector (④) into the waste tank.

Performing Daily Inspection

Before running any samples on the bioanalyzer, perform the following:

- Verify the connectors on the fluidic tanks are properly connected.

- Open the filter compartment door and verify that the particulate filters are properly secured. See Filter Replacement on page 311 for more information.

- Follow the instructions in Leak Detection on page 307.

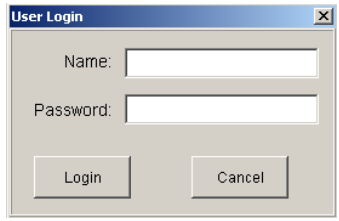
Starting Up the Workstation and Software

1 Start up the workstation.

2 Log into Windows.

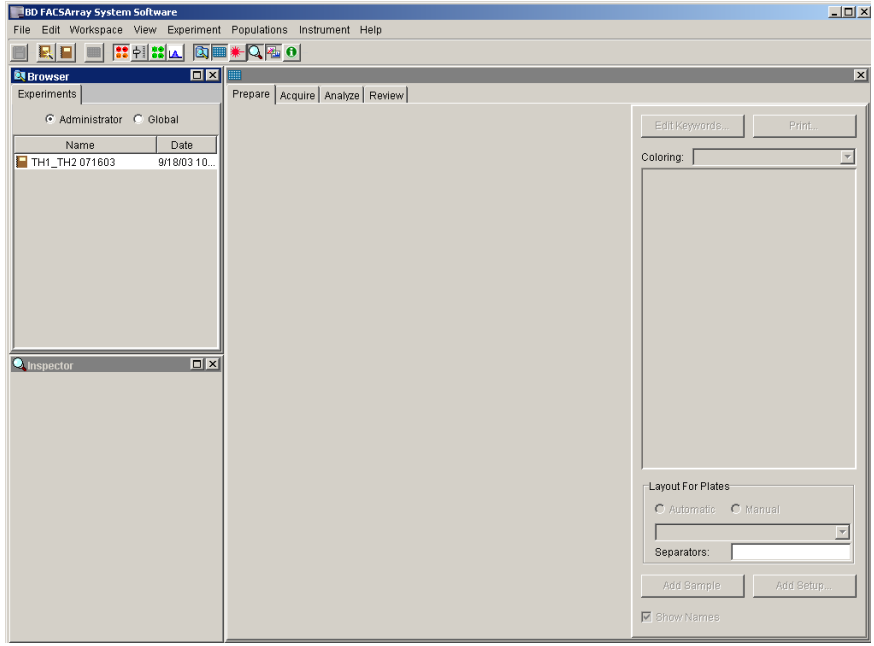
3 Double-click the BD FACSArray system software icon on the desktop to start the software.

4 Enter your user Name and Password in the User Login dialog.



The Prepare workspace appears (Figure 5-3 on page 147).

Figure 5-3 Default Prepare workspace



5 Before performing instrument QC, allow the laser to warm up for 20 minutes after powering up the bioanalyzer.

Performing Instrument Quality Control

Instrument quality control (QC) is a process that ensures consistent instrument performance on a daily basis. Performing instrument QC consists of preparing the instrument and software, running QC particles (usually beads), and saving the QC results. Performing QC with identical instrument settings on a daily basis and monitoring the change in the means and CVs of the populations can alert you to changes in the BD FACSArray bioanalyzer's performance.

Perform instrument QC daily prior to running samples on the bioanalyzer. You can use a separate plate for running the QC particles or add extra setup wells to the beginning of your sample plate.

Selecting QC Particles

Choose particles for QC purposes that are stable over a long period of time and monitor performance characteristics that are important to you. A set of particles that monitors a wide range of performance characteristics, including sensitivity and resolution, is preferable. An example of a recommended QC sample would include particles that are non-fluorescent, dimly fluorescent, and brightly fluorescent in each of the fluorescence detectors.

⚠ **NOTICE** To avoid damaging the BD FACSArray bioanalyzer, do not select QC particles greater than 50 µm in diameter.

Creating the Experiment

The simplest way to acquire and analyze QC data is to customize the default QC template for your bioanalyzer. Once you have a customized template, use the Experiment Wizard to create a new experiment from your template and collect all data in that experiment. Using this method, all QC samples will be collected with the same instrument settings and appear in the same gates, making it simple to review the data and detect changes in your bioanalyzer's performance.

The default QC template was created for use with a set of QC particles that appear as dim, medium, and bright populations in all fluorescent parameters. The template can be further customized for use with alternate particles.

The first time you run QC particles, you will have to create a new experiment. For subsequent runs, you can open and reuse the same experiment.

Opening the Default QC Template

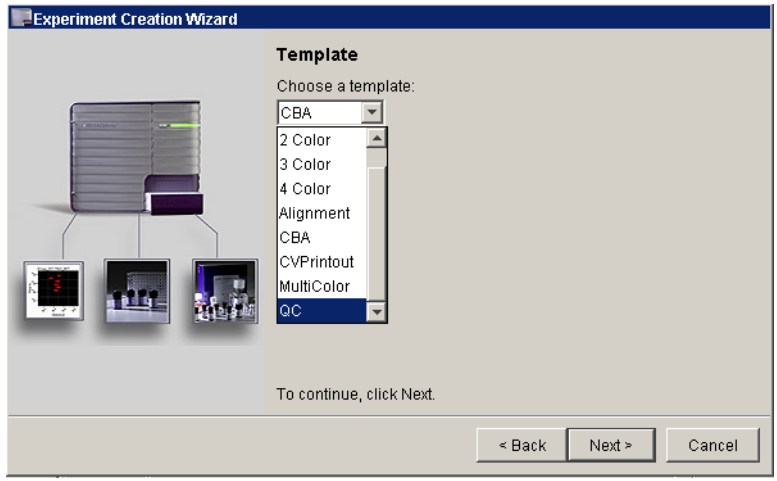
1. Click on the Experiment Wizard tool ( ) in the application toolbar.

   Alternatively, you can choose Experiment > Experiment Wizard.

   The Welcome view appears. After a few seconds, the Selecting a Wizard Session view appears.

2. Choose Default from the Wizard Session list; click Next.

3 In the Template view, select QC from the template drop-down menu; click Next.



Entering Sample Information

1 In the Instrument Setup and Optical Spillover view, click No if it's not already selected; click Next.

2 In Number of Samples view, enter 8; click Next.

3 In the Sample Identification view, click Next to accept the default names.

4 Choose the default number of wells in the Number of Wells Per Sample view; click Next.

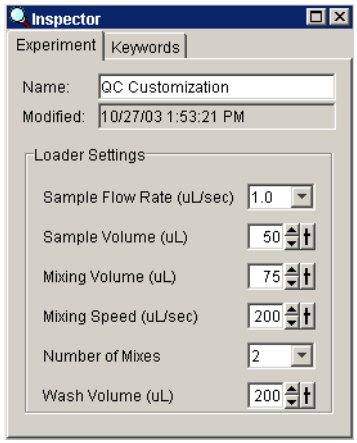
5 In the Well Names view, click Next to accept the default names; click Next.

6 In the Fluorophore Labels view leave labels blank and click Next.

7 In the Custom Keywords view, click Next.

8 Choose the default plate layout; click Next.

9 In the Loader Settings view, change the sample flow rate to 0.5 µL/sec and the sample volume to 100 µL; click Next.

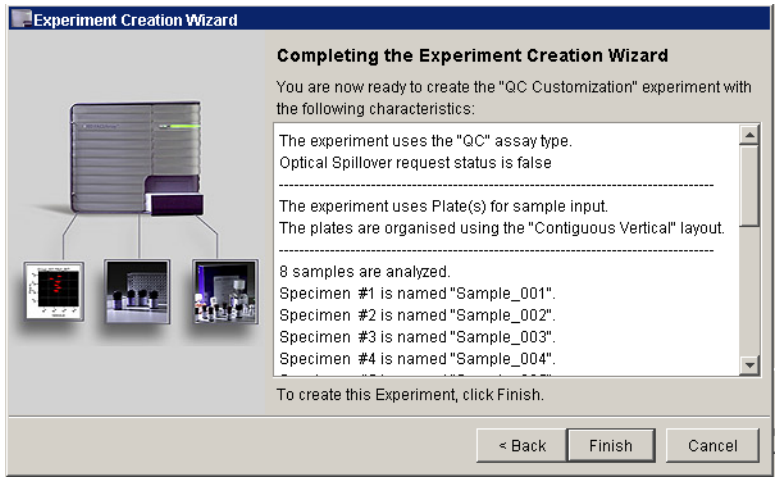


10 In the Acquisition Settings view, leave the default settings; click Next.

11 Enter *QC Customization* in the Experiment Name view; click Next.

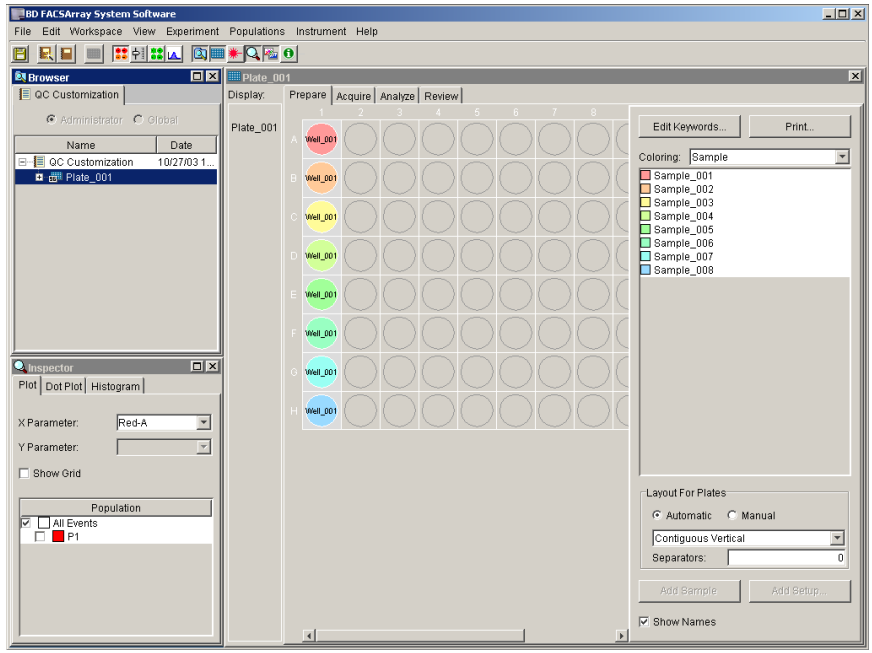
12 In the Saving Wizard Session view, choose not to save the session; click Next.

13 Review the information you entered in the Completing the Experiment Wizard view using the scroll bar to view the entire summary.


Experiment Creation Wizard
Completing the Experiment Creation Wizard
You are now ready to create the "QC Customization" experiment with the following characteristics:
The experiment uses the "QC" assay type.
Optical Spillover request status is false
The experiment uses Plate(s) for sample input.
The plates are organised using the "Contiguous Vertical" layout.
8 samples are analyzed.
Specimen #1 is named "Sample_001".
Specimen #2 is named "Sample_002".
Specimen #3 is named "Sample_003".
Specimen #4 is named "Sample_004".
To create this Experiment, click Finish.
< Back
Finish
Cancel

14 After reviewing the summary information, click Finish.

There will be a slight pause as the experiment is created, and then the Prepare workspace appears.



Preparing the Plate

1 Prepare the QC particles according to the manufacturer's instructions.

2 Pipette QC particles into the first eight wells of a plate.

Loading the Plate

⚠ **CAUTION** Movement of mechanical parts within the instrument can pinch or injure your hands or fingers. To prevent injury by moving parts, follow these precautions.

- Keep your hands away from the plate holder once you position the plate on it. The plate holder will automatically retract when you click Load.
- Keep your hands away from the plate door during instrument operation.

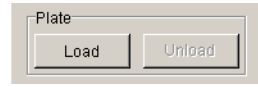
⚠ **NOTICE** To prevent damage to the bioanalyzer, remove any lid, film, or cover from the plate before loading onto the plate holder. Some combinations of plate and lid might not retract properly into the instrument, potentially causing damage to the plate and the plate sampler. Leaving a lid, film, or cover on the plate might cause clogs in the probe and sample line, affecting performance and possibly damaging the bioanalyzer.

1 Place the plate on the plate holder.

Line up well A1 on the plate with the A1 mark on the plate holder.

2 Click the Setup tool ( ) in the application toolbar to open the Setup workspace.

3 Click the Load button in the Plate Control frame.



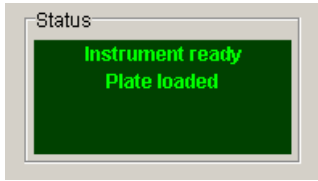
The plate will be retracted three-quarters of the way into the bioanalyzer.

- If a lid is detected on the plate, the plate will be ejected from the bioanalyzer, and the following message will appear on the computer monitor.

Remove the lid and click the Load button in the Plate Control frame to reload the plate.

- If no lid is detected on the plate, the plate is completely retracted into the bioanalyzer.

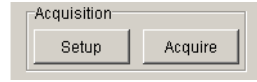
The Status box should display the following message.



Adjusting the Settings

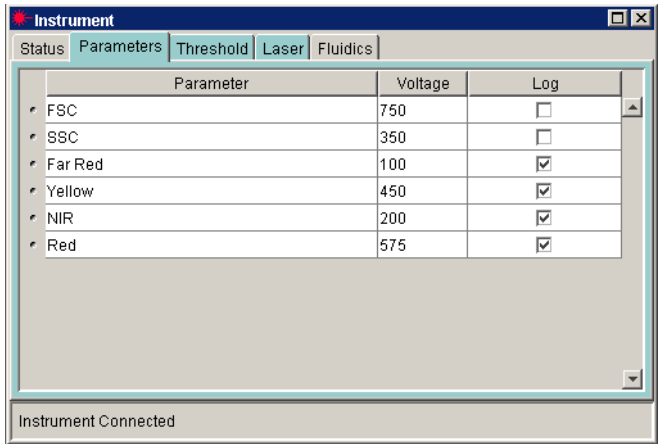
1 In the plate view, select well A1.

2 Click Setup in the Acquisition Control frame.



Events appear in the plots, but data is not saved.

3 If not already selected, click the Parameters tab in the Instrument frame to view parameter voltages.



4 Adjust the FSC and SSC voltages to put the QC particles on scale.

5 In the Instrument frame, click the Threshold tab to open it.

6 Adjust the FSC threshold in the FSC vs SSC dot plot to exclude noise.

7 Click the Parameters tab to again view parameter voltages.

8 While observing the first histogram in the Template view, adjust the Far Red-A fluorescent voltage to put the QC particles on scale.

It can be useful to set all populations to appear in a similar channel in each histogram. However, this might not be possible depending on the particles you are using.

NOTICE If the sample in well A1 runs out before you have finished optimizing the settings, do the following:

- Select well A2 on the plate view.
- Click Setup to begin acquisition.

9 Repeat step 8 for the rest of the parameters while viewing the corresponding histogram.

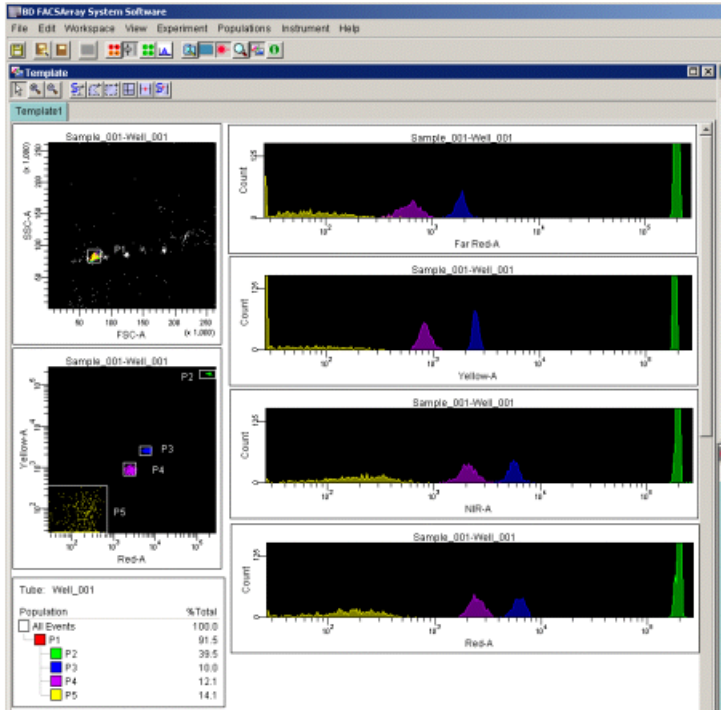
10 Create gates to enclose each population.

Gate statistics appear in the Statistics view.

For information on creating gates, see Gates on page 117.

11 Click Setup to stop acquisition.

The following figure shows an example of data obtained from QC particles with unstained, dim, medium, and bright populations.



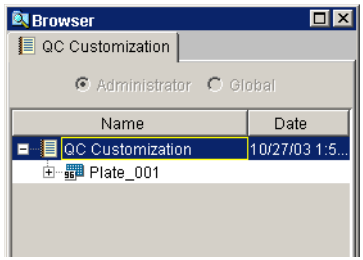
12 Click Unload and remove plate from plate sampler.

Customizing the Loader Settings for Daily QC

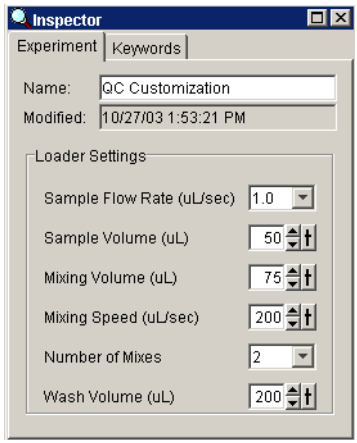
You have established the gates and instrument settings for daily QC. Before saving as a customized template, optimize loader settings for sample volume and flow rate for your QC application.

1. Click the Prepare tool ( ) in the application toolbar to open the Prepare workspace.

2. In the Browser, click on the experiment name to select it.



3. In the experiment Inspector, review the loader settings.



4 Adjust the settings to be useful for your daily QC particles. Take into consideration the concentration of the sample and the sample flow rate you want to achieve.

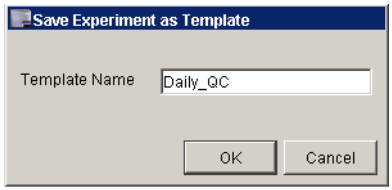
For more information on loader settings, see Inspector on page 103. For information on adjusting loader settings, see Preparing for Optimization on page 225.

Saving the Experiment as a Template

1 Choose Experiment > Save As Template.

2 Enter the name of the template in the text field.

For this example, name the template *Daily_QC*.



3 Click OK to save the template.

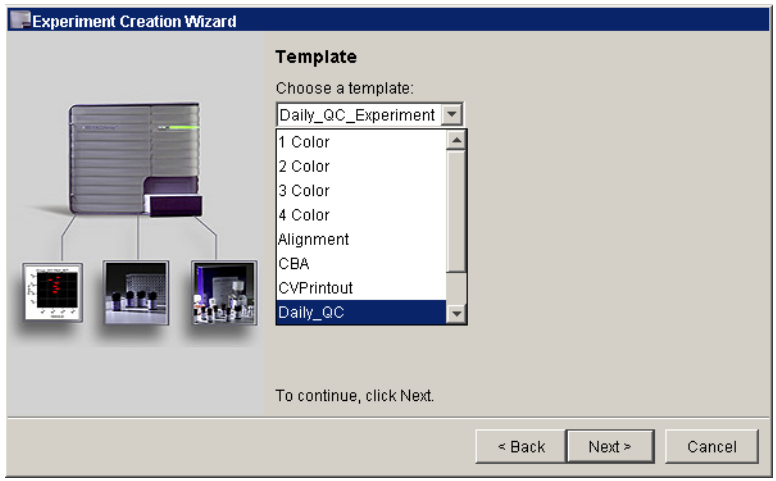
Verifying the Template

Creating a New Experiment

1 Open the Experiment Wizard by clicking the Experiment Wizard tool ( ).

2 Choose the Default Wizard session; click Next.

3 In the Template view, select the *Daily_QC* template you just created from the template drop-down menu; click Next.



4 In the Instrument Setup and Optical Spillover view, click No if it's not already selected; click Next.

5 In the Number of Samples view, enter 96; click Next.

6 In the next five views, use the default settings; click Next.

7 Choose a convenient plate layout; click Next.

8 Accept the default loader settings; click Next.

9 In the Acquisition Settings view, choose settings that are consistent with your QC particle concentration, sample volume, and flow rate; click Next.

10 Enter a name for your experiment in the Experiment Name view; click Next.

For this example, enter *Daily_QC_Experiment*.

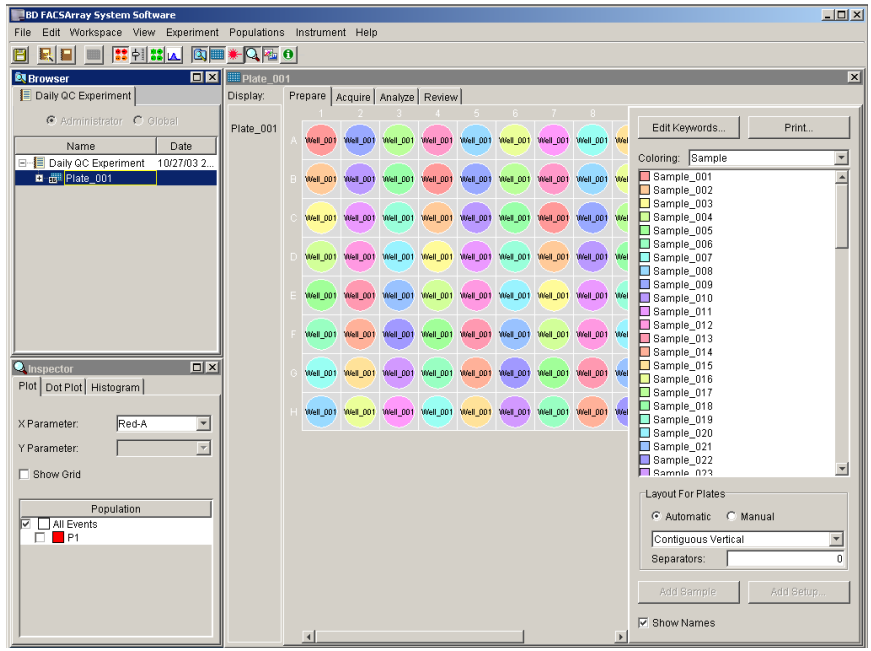
11 In the Saving Wizard Session view, choose whether to save the session and enter a session name; click Next.

For this example, click the Yes radio button to save the session, click in the name field, and enter *Daily_QC_Experiment_Wizard* as the session name.

12 In the Completing the Experiment Wizard view, click Finish.

There will be a slight pause as the experiment is created.

The new experiment appears in the Prepare workspace.



Collecting Daily QC Data

☑ **Tip** To simplify collection and analysis of daily QC data, use a single 96-well plate to collect 96 days of QC data. Load the wells sequentially each day. Store the plate at room temperature covered with a lid to prevent contamination.

1 Prepare QC particle according to the manufacturer's instructions.

If required, prepare a fresh QC particles suspension.

2 Pipette 200 µL QC particles suspension into well A1 of a new 96-well plate.

Loading the Plate

⚠ **CAUTION** Movement of mechanical parts within the instrument can pinch or injure your hands or fingers. To prevent injury by moving parts, follow these precautions.

- Keep your hands away from the plate holder once you position the plate on it. The plate holder will automatically retract when you click Load.
- Keep your hands away from the plate door during instrument operation.

⚠ **NOTICE** To prevent damage to the bioanalyzer, remove any lid, film, or cover from the plate before loading onto the plate holder. Some combinations of plate and lid might not retract properly into the instrument, potentially causing damage to the plate and the plate sampler. Leaving a lid, film, or cover on the plate might cause clogs in the probe and sample line, affecting performance and possibly damaging the bioanalyzer.

1 Click the Setup tool ( ) in the application toolbar to open the Setup workspace.

2 Place the plate on the plate holder.

Line up well A1 on the plate with the A1 mark on the plate holder. See Figure 5-4 on page 161.

Figure 5-4 Plate holder showing A1 mark

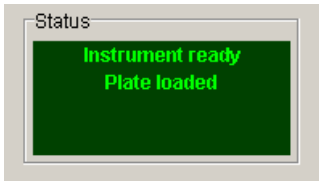
3 Click the Load button in the Plate Control group.

The plate will be retracted three-quarters of the way into the bioanalyzer.

- If a lid is detected on the plate, the plate will be ejected from the bioanalyzer, and the following message will appear on the computer monitor.

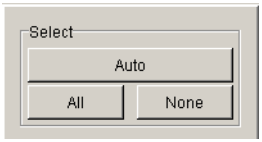
- If no lid is detected on the plate, the plate is completely retracted into the bioanalyzer.

The Status box should display the following message.



Verifying the Template Settings

1 In the plate view, click None in the Select Control group.



2 Click on well A1 to select it.

3 Click Setup to start viewing data.

4 In the Template view, verify that the QC particles are enclosed by any gates you created.

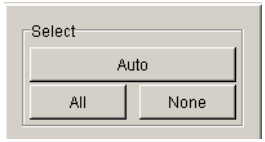
If the template was customized successfully, the populations should fall within the established gates.

5 Click Setup to stop viewing data.

⚠ **NOTICE** The parameter voltages required to place QC particles in specific channels are determined by the characteristics of the bioanalyzer optics, detectors, electronics, and fluidics. Repairs or major maintenance to the bioanalyzer might require adjustments to the parameter voltages used in all experiments, including the Daily QC Experiment. BD Biosciences strongly recommends running QC particles after field service repairs or maintenance to determine appropriate changes to the parameter voltage settings. All QC data from that point forward should be acquired with the new settings.

Acquiring QC Data

1 In the plate view, click None in the Select Control group.



2 Click well A1 to select it.

3 Click Acquire.

Data will be saved. When you have acquired the correct number of events or the sample is exhausted, acquisition will stop.

4 Click Unload and remove the plate from the plate sampler.

Printing Experiment Information

If you want to save a copy of your data or the Instrument Status Report, use the following procedure.

1 Print the Template view by choosing File > Print.

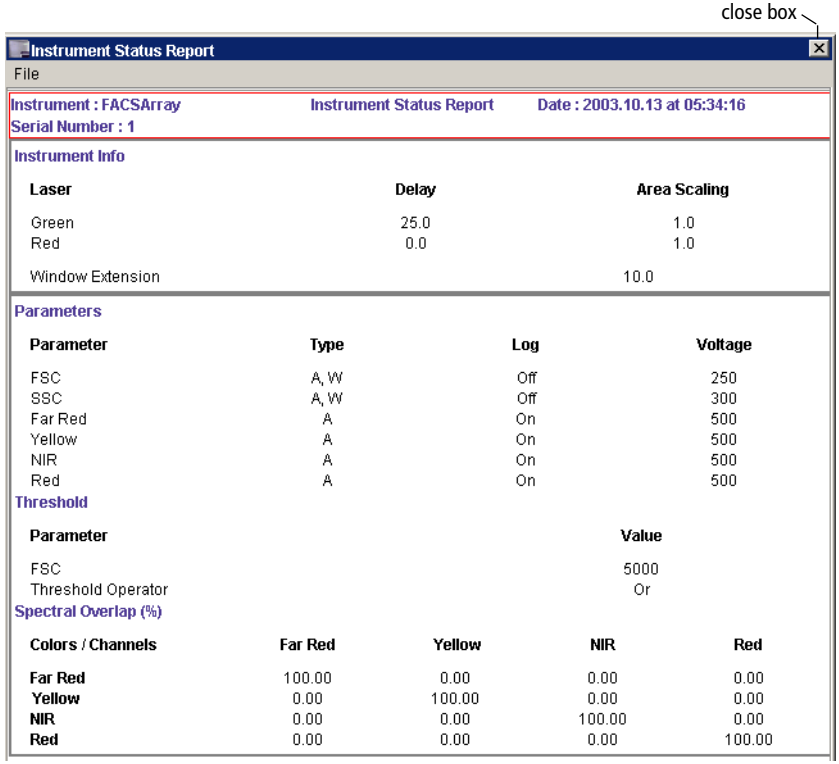
A print dialog appears.

2 Click OK to print the Template view.

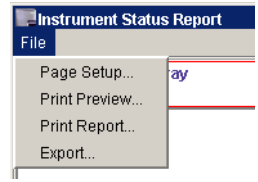
3 Choose Instrument > Instrument Status Report.

The Instrument Status Report appears. See Figure 5-5 on page 164.

Figure 5-5 Instrument Status Report



4 Click on the Instrument Status Report, open the file menu on the report, and choose Print Report.



A print dialog appears.

5 Click OK to print the report.

6 Close the report by clicking the close box on the Instrument Status Report (Figure 5-5).

☑ **Tip** To track bioanalyzer performance, retain a copy of the Instrument Status Report and the printout of the Template view for each QC run.

7 Remove the plate from the plate holder and store the plate at room temperature covered with a lid.

8 To collect QC data for the next day, repeat this procedure except add QC particle suspension to well A2.

Analyzing QC Data

To efficiently analyze QC data, accumulate data from several days, and then perform the following.

1 Review the acquired wells in the Analyze workspace.

2 Adjust gates to capture the appropriate populations.

3 Batch export the QC statistics.

See Exporting Statistics on page 137 for instructions.

4 Open the exported statistics file in a spreadsheet software program, such as Microsoft Excel.

☑ **Tip** To see the exported statistics file name when opening the file in the spreadsheet program, choose All Files (*.*) from the File Type drop-down menu.

5 Create graphs to track the mean and CV of each QC particle population over time.

6 Update the graphs periodically to monitor bioanalyzer performance.

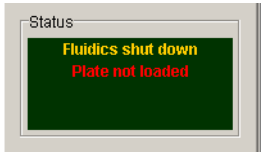
Shutting Down the Instrument

Perform the following procedure at the end of the day before turning off the bioanalyzer.

1. Perform Daily Cleaning on page 298.

2. Choose Instrument > Shutdown Fluidics.

   Do **not** turn off the bioanalyzer until the following message appears in the Status box.



3. Turn off the bioanalyzer by pressing the power switch on the upper front panel of the instrument.

6

CBA Analysis

This chapter includes a tutorial that will guide you through experiment setup, acquisition, and analysis of a BD Cytometric Bead Array (CBA) assay on the BD FACSArray bioanalyzer.

The following topics are covered in this chapter.

- Workflow Overview on page 169
- Creating a BD CBA Experiment Using the Wizard on page 169
- Preparing for Acquisition on page 180
- Acquiring Samples on page 185
- Reacquiring Samples on page 191
- Analyzing BD CBA Data on page 192
- Running Multiple CBA Assays on One Plate on page 193

The BD FACSArray provides fast, sensitive, plate-based analysis of cellular proteins. When secreted or present in cell lysates, cellular proteins can be detected and quantified with the multiplexed fluorescent BD Cytometric Bead Array (BD CBA) technology.

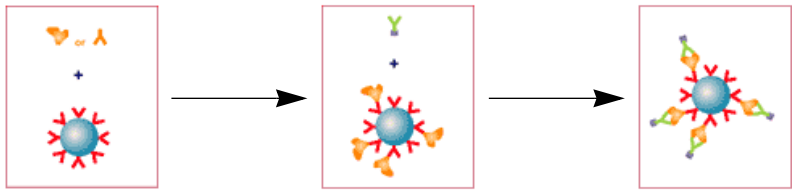
Some of the applications compatible with the bioanalyzer include:

- Cytokine and chemokine profiling
- Inflammatory response
- Apoptosis
- Cell signaling events

The BD CBA assay is commonly referred to as a multiplexed bead assay. A series of beads with discrete fluorescence intensities are used to simultaneously detect multiple analytes in a small sample volume. The beads serve to capture and quantify soluble analytes.

Figure 6-1 shows the basic sandwich assay schema for the BD CBA. Analytes in samples bind to a bead with a given fluorescent intensity that is coated with capture antibodies specific for a particular analyte (left). A reporter antibody (antibody attached with fluorescent molecules such as phycoerythrin [PE] that is different from the capture antibody), binds to the analyte (center). Unbound (excess) reporter antibodies and analytes are eliminated by washing (right).

Figure 6-1 Sandwich schema for BD CBA



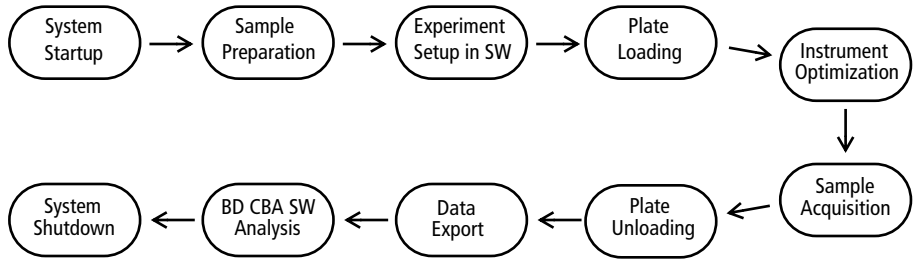
Each bead population shown in the Red-A histogram is distinguishable by different amounts of red fluorescence and is coated with capture antibodies for a specific analyte. In the Yellow-A vs Red-A dot plot, yellow fluorescence intensity (PE) indicates the amount of bound analyte.

The analytes are then measured by the bioanalyzer. BD FACSArray system software is used to acquire and export data as FCS files that are readily accessible to BD CBA software for analysis.

Workflow Overview

Figure 6-2 shows you an overview of the steps required to process and run samples on the BD FACSArray bioanalyzer. Use this overview to help you structure workflow for your applications.

Figure 6-2 Workflow overview



Creating a BD CBA Experiment Using the Wizard

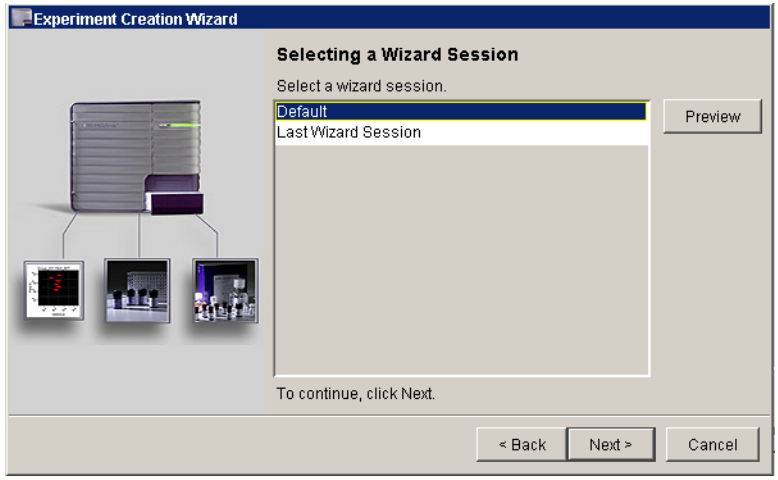
Use the following tutorial to help you create a BD CBA experiment with the Experiment Wizard. This example is based on the BD CBA Human Th1/Th2 Cytokine assay.

⚠ **NOTICE** Be sure that your sample layout on the plate matches what you set up using the Experiment Wizard. Sample layout created by the Experiment Wizard cannot be rearranged to match a pre-loaded plate.

Choosing a Wizard Session

1 Click the Experiment Wizard tool ( ) in the application toolbar.

The Experiment Wizard Welcome dialog appears. This view remains for approximately 2 seconds and then automatically advances to the next view.



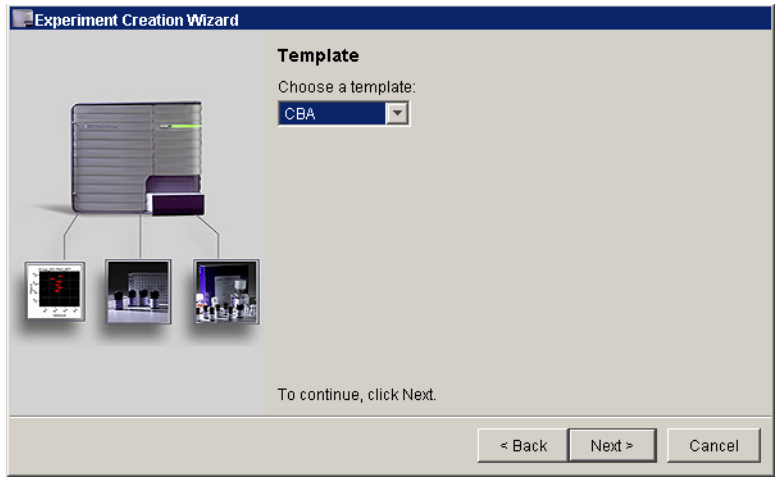
If you want to quit the Experiment Wizard session without saving the experiment, click the Cancel button on any of the Wizard views.

2 Choose the Default Wizard session; click Next.

3 In the Template view (Figure 6-3 on page 171), click the template drop-down menu and select CBA if not already selected; click Next.

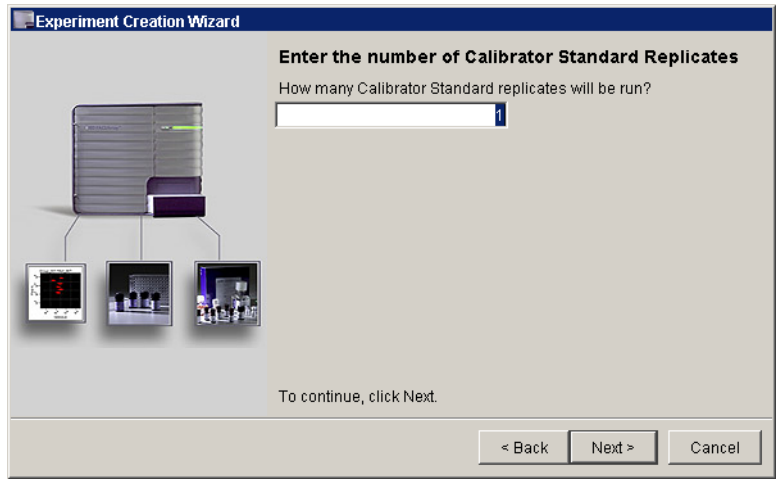
The CBA template contains predefined plots, a gate, statistics, and loader and instrument settings that serve as starting points for sample setup and optimization for CBA applications.

Figure 6-3 Template view



Entering Standard and Sample Information

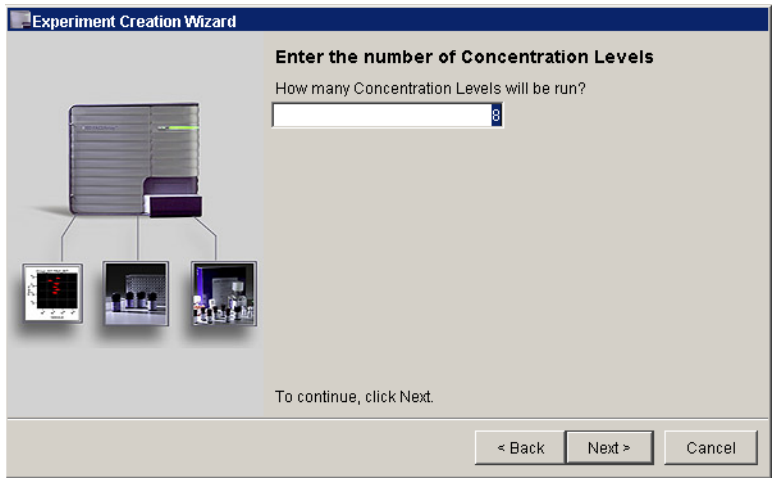
1 Use the default value of 1 for the number of calibrator standard replicates; click Next.


Experiment Creation Wizard
Enter the number of Calibrator Standard Replicates
How many Calibrator Standard replicates will be run?
1
To continue, click Next.
< Back
Next >
Cancel

2 Use the default number of concentration levels (8); click Next.

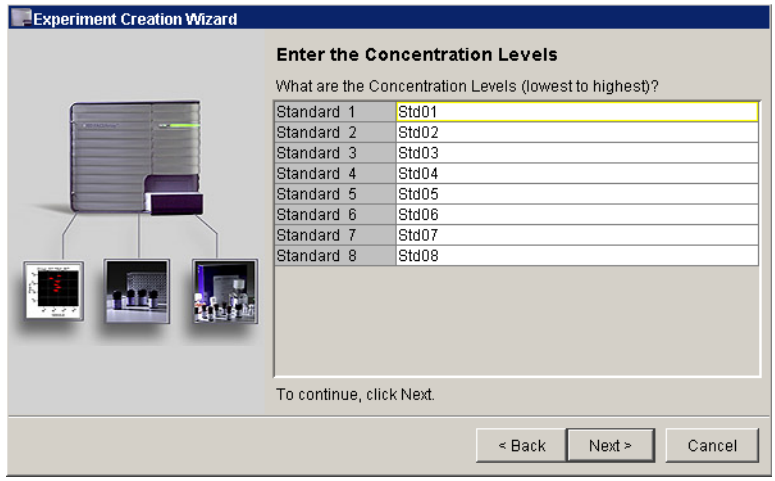
You can change the concentration levels to any number you want. Using eight is convenient because it takes up one column of a plate when running in a vertical mode.

If necessary, you can add additional standards and run them 12 across using a row-based plate layout. See Layout for Plates Control Group on page 74 for more information about plate layout.



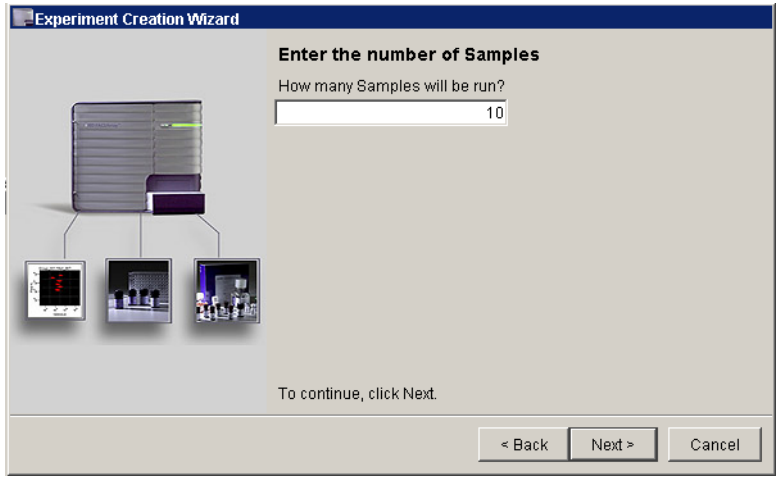
3 Use the default concentration level names (Figure 6-4 on page 173); click Next.

Figure 6-4 Concentration Levels view

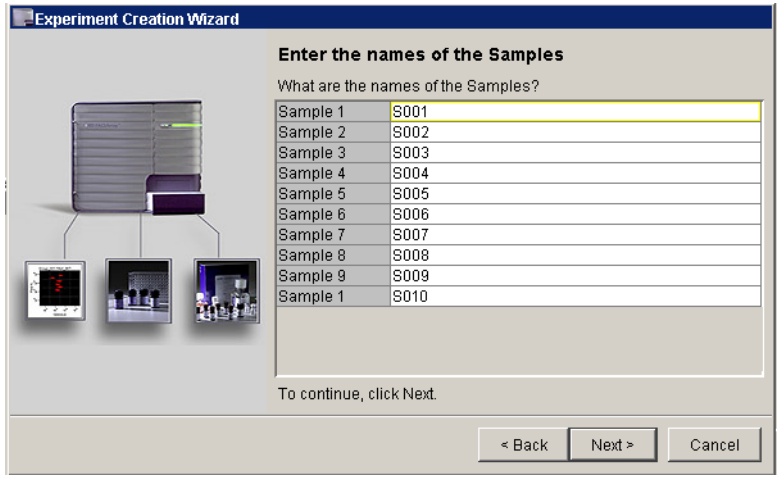


4 Enter 10 for the number of samples to be run; Click Next.

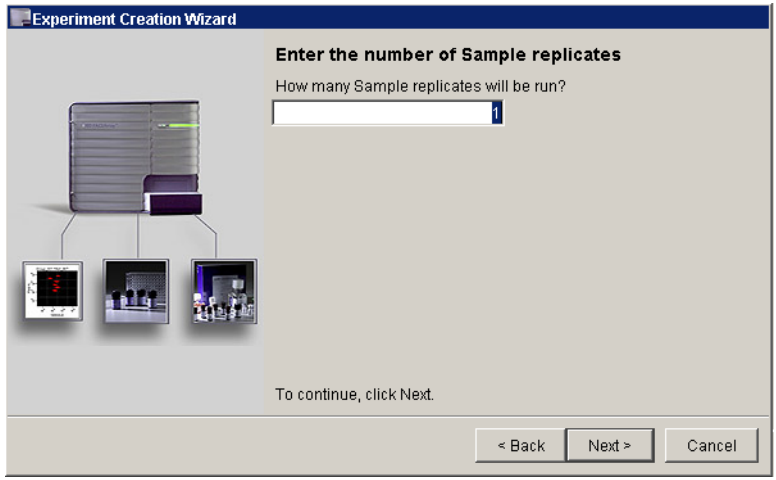
BD FACSArray system software has no limitation on the number of samples or plates per experiment that you can run. However, BD CBA software can analyze up to five plates per experiment.



5 Use the default sample names; click Next.



6 Use the default setting of 1 for the number of sample replicates; click Next.



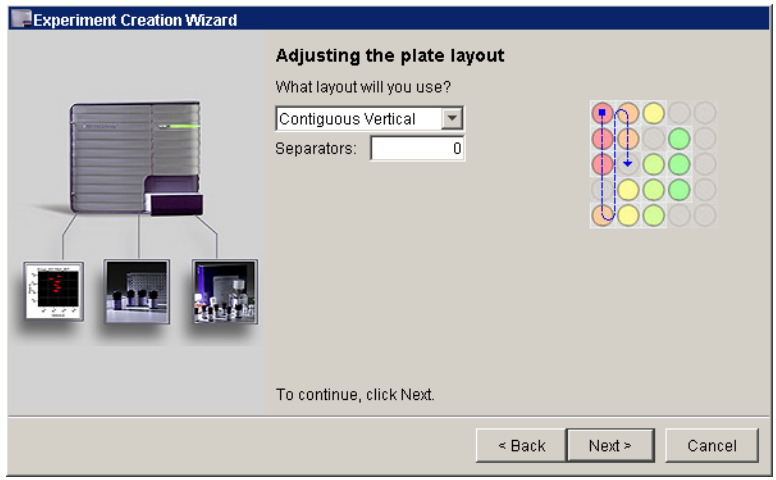
☑ **Tip** You can use additional replicates if you want to generate precision data.

Choosing Additional Experiment Criteria

1 Choose the default settings of Contiguous Vertical with 0 Separators (blank wells to add between samples); click Next.

Separators are blank wells you can add between samples.

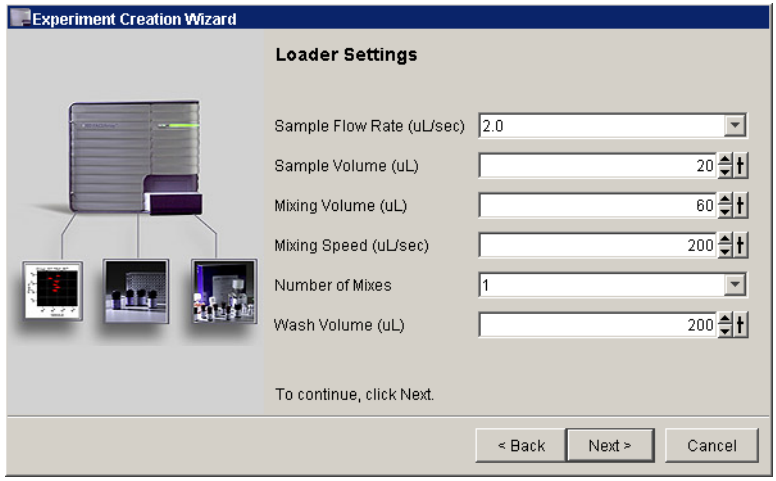
In this view, you can choose how to lay out samples on the plate. See Layout for Plates Control Group on page 74 for more information about adjusting the plate layout.



NOTICE You can choose Contiguous Horizontal if you want to set up and run the plate horizontally.

2 Use the default loader settings (Figure 6-5 on page 176); click Next.

Figure 6-5 Loader Settings view

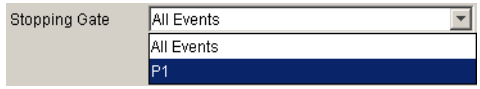


The default loader settings in this CBA experiment template are specific for BD CBA assays. BD recommends using the default settings if you are running samples prepared with BD CBA assay kits.

3 In the Acquisition Settings view, choose the following options if they are not already selected.

- Click in the Events to Acquire drop-down menu, enter 1200 evt, and then press the Enter key on your keyboard.

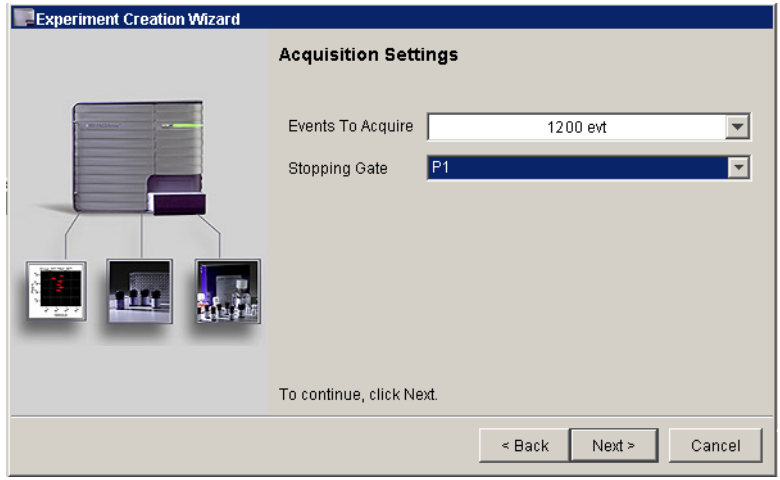
- Click the Stopping Gate drop-down menu and choose P1 as the stopping gate.



Acquisition will stop when there are 1200 events in P1.

In the BD CBA template, an acquisition gate has been predefined. You will use the predefined gate (P1) as a stopping gate. A stopping gate triggers acquisition to stop when the specified number of events chosen in the Events to Acquire field has been reached.

The default number of events is set to 1200 and is recommended for 6-bead assays such as Human TH1/TH2 Cytokine assay.



☑ **Tip** If you are running a single bead assay, adjust the Events to Acquire to 200.

4 Click Next.

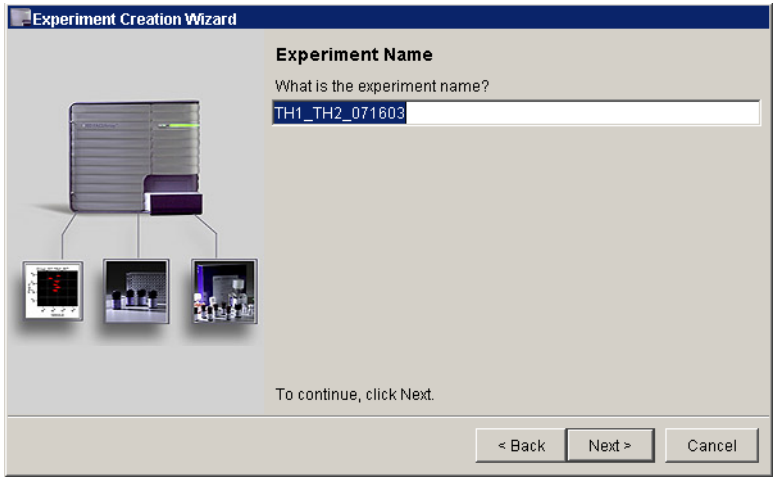
Naming the Experiment and Wizard Session

1 Enter the name as *TH1_TH2_071603* (Figure 6-6 on page 178); click Next.

The experiment name is derived from the assay name, which is TH1 TH2, and the date.

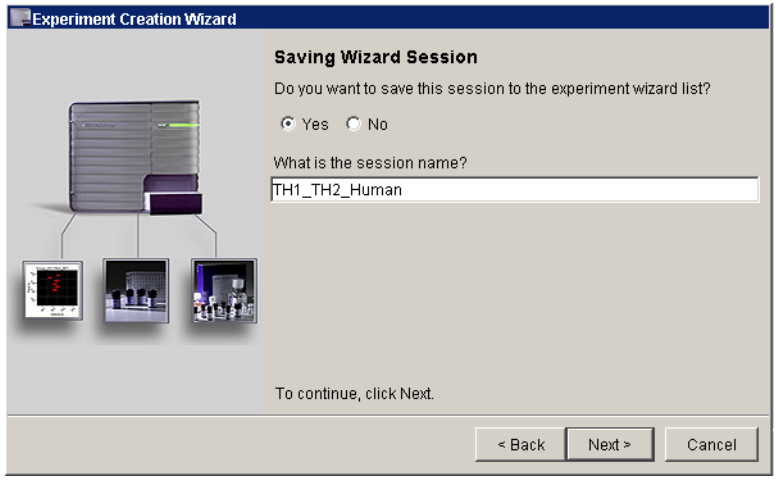
☑ **Tip** Locate saved data more easily by naming experiments and experiment elements with meaningful names.

Figure 6-6 Experiment Name view



2 Click the Yes radio button and enter the name *TH1_TH2_Human*; click Next.

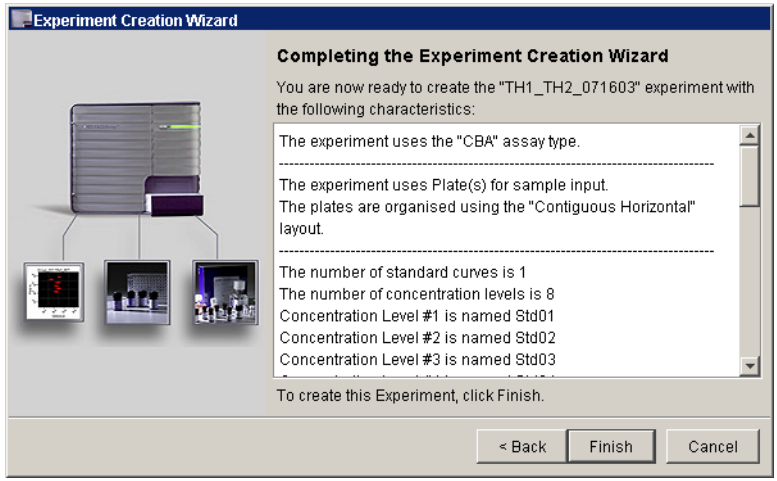
Saving the Wizard session allows you to reuse the session for other similar experiments, and allows you to modify any of the existing settings.



NOTICE If you prefer, you can use the same name for the Wizard session as you did for the experiment.

Reviewing the Wizard Session

1 In the Experiment Wizard summary view, review the choices you have made.


Experiment Creation Wizard
Completing the Experiment Creation Wizard
You are now ready to create the "TH1_TH2_071603" experiment with the following characteristics:
The experiment uses the "CBA" assay type.
The experiment uses Plate(s) for sample input.
The plates are organised using the "Contiguous Horizontal" layout.
The number of standard curves is 1
The number of concentration levels is 8
Concentration Level #1 is named Std01
Concentration Level #2 is named Std02
Concentration Level #3 is named Std03
To create this Experiment, click Finish.
< Back
Finish
Cancel

Use the scroll bar to see the entire view.

If you want to change any of the options you chose, click the Back button until you reach the appropriate page, make the change, and then click the Next button until you reach the summary page.

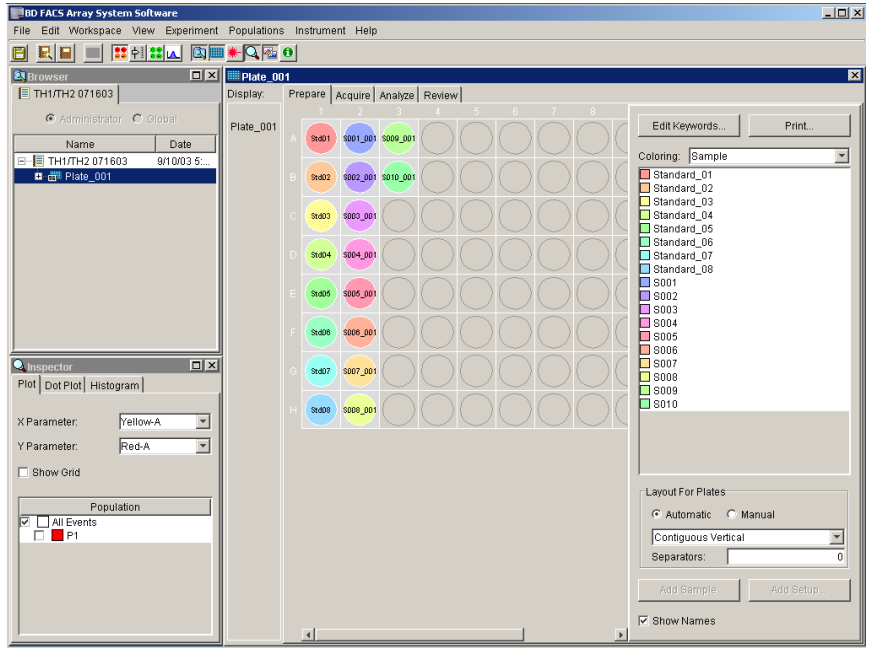
☑ **Tip** Print the information in the summary view by selecting the text in the summary box, pressing Ctrl-C on your computer keyboard, pasting the information into Microsoft Word or Wordpad, and printing the document.

2 Click Finish.

The experiment you just created opens in the Prepare workspace (Figure 6-7 on page 180).

Depending on the size of the experiment (number of sample wells and number of plates), it might take several minutes for the Prepare workspace to appear.

Figure 6-7 Prepare workspace showing newly created experiment



Preparing for Acquisition

Preparing the Samples

There are a wide variety of BD CBA kits available. The kits provide reagents for staining samples, calibrator standards, and a manual with step-by-step instructions on how to prepare samples in 12 x 75-mm tubes. This section describes how to prepare CBA samples in 96-well filter-bottom plates.

Materials

See Consumables on page 359 for ordering information.

- Digital stirrer
- Filter plates
- Vacuum manifold
- Multichannel pipette (8- or 12-channel)
- Vacuum source

Filter Plate Assay Protocol

Performing a CBA experiment in a 96-well filter plate is very similar to the protocol described in the BDA kit manual. Follow all information in the manual for the CBA kit being assayed. The following plate-specific instructions for samples run on the BD FACSArray bioanalyzer are the only modifications to the CBA assay protocol described in the CBA kit manual.

⚠ **NOTICE** Be sure that your sample layout on the plate matches what you set up using the Experiment Wizard. Sample layout created by the Experiment Wizard cannot be rearranged to match a pre-loaded plate.

☑ **Tip** Use a printout of the plate view as a guide for transferring samples to the 96-well plate. To print the plate view, click the Print button in the upper right corner of the Prepare workspace.

1. Add reagents to the filter plate according to the CBA kit manual.

   Add beads, detection reagents, and sample in the same volumes and order as described in the CBA kit manual.

2. Place the cover or lid on the plate.

3 Put the prepared plate on a plate shaker for 5 minutes at 1,100 rpm.

This ensures reagents and samples are well-mixed.

4 Incubate the plate as described in the Assay Protocol in the CBA kit manual.

5 Remove the plate cover.

6 Apply the plate to the vacuum manifold and vacuum aspirate until the wells are drained (5–10 seconds).

⚠ **NOTICE** Do not exceed 10 in. Hg of vacuum.

7 If you are staining serum samples, perform the following; if you are not staining serum samples, skip to step 8.

- Add 200 µL of wash buffer to each well.
- Apply the plate to the vacuum manifold and vacuum aspirate until the wells are drained (5–10 seconds).

8 After final assay wash, add 120 µL of wash buffer to each well.

This step resuspends the beads.

9 Put the prepared plate on a plate shaker for 2 minutes.

10 Immediately run the plate on the BD FACSArray bioanalyzer.

Loading the Plate

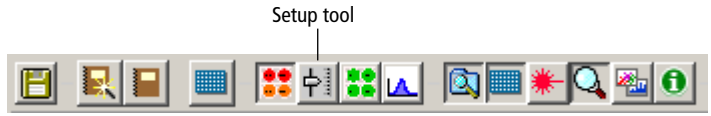
⚠ **CAUTION** Movement of mechanical parts within the instrument can pinch or injure your hands or fingers. To prevent injury by moving parts, follow these precautions.

- Keep your hands away from the plate holder once you position the plate on it. The plate holder will automatically retract when you click Load.

- Keep your hands away from the plate door during instrument operation.

⚠ **NOTICE** To prevent damage to the bioanalyzer, remove any lid, film, or cover from the plate before loading onto the plate holder. Some combinations of plate and lid might not retract properly into the instrument, potentially causing damage to the plate and the plate sampler. Leaving a lid, film, or cover on the plate might cause clogs in the probe and sample line, affecting performance and possibly damaging the bioanalyzer.

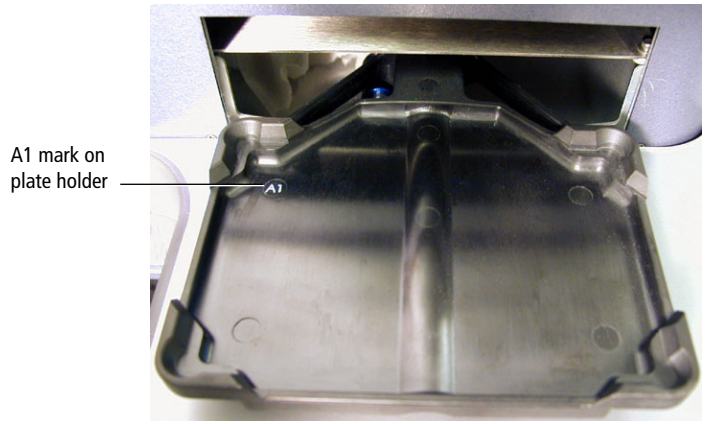
1 Click the Setup tool ( ) in the application toolbar to open the Setup workspace.



The wells you selected for acquisition appear in the plate view. The wells are numbered in acquisition order and each sample is color-coded.

2 Place the plate containing the stained samples on the plate holder.

Position the plate so that well A1 is over the A1 mark on the plate holder.



3 Click the Load button in the Plate Control frame.

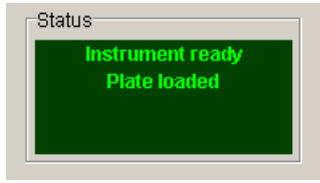
The plate will be retracted three-quarters of the way into the bioanalyzer.

- If a lid is detected on the plate, the plate will be ejected from the bioanalyzer, and the following message will apper on the computer monitor.

Remove the lid and click the Load button in the Plate Control frame to reload the plate.

- If no lid is detected on the plate, the plate is completely retracted into the bioanalyzer.

The Status box should display the following message.



Verifying the Gate

1. In the Select Control group in the Plate Editor, click None to deselect all wells in the plate view.
2. Click the first well.
3. Click Setup in the Acquisition Control group.

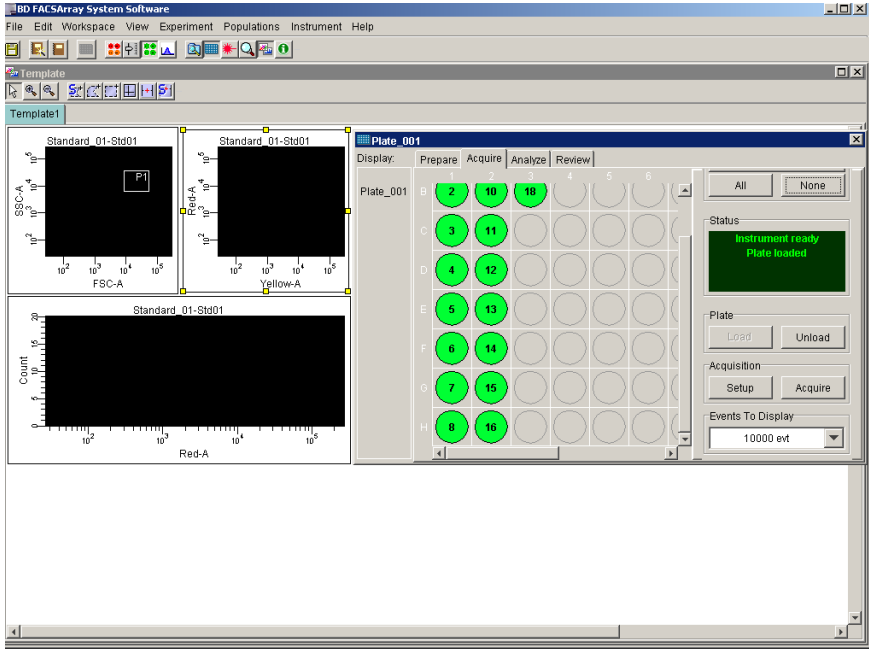
   Events appear in the plots, but data is not being saved.
4. Verify:

   - P1 gate on the FSC vs SSC plot encloses the entire singlet population. If necessary, move or resize the P1 gate to enclose the singlet population.
   - All six bead populations appear on the histogram. If you do not see all six populations on the histogram, go to Troubleshooting on page 337 for instructions.
5. Click Setup to stop acquisition.

⚠ **NOTICE** To avoid depleting sample in the first well, minimize sample use for verification.

Saving Data

1 Click the Acquire tool ( ) in the application toolbar to open the Acquire workspace.



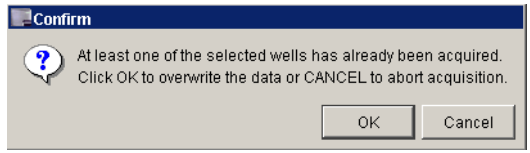
2 Click the Acquire button in the Acquisition frame.

After a short pause, the following occurs.

- An orange ring appears in the first well selected for acquisition indicating that sample is being acquired.

- Events appear in the plots.
- Data is saved.

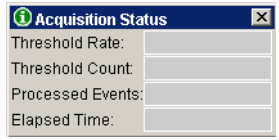
If any of the wells selected for acquisition already contain data, an alert dialog appears.


Confirm
At least one of the selected wells has already been acquired.
Click OK to overwrite the data or CANCEL to abort acquisition.
OK
Cancel

Clicking OK will begin acquisition and data will be overwritten. Clicking Cancel will abort acquisition.

What to Monitor During Acquisition

1. Click the Acquisition Status tool ( ) in the application workspace to open the Acquisition Status frame.



The Acquisition Status frame is a useful tool to monitor whether events are being detected and processed during acquisition.

2. Monitor the Status box for error messages.

If an instrument error occurs during acquisition, a message will be displayed on the Status frame in the Acquire workspace.

3. To view error information, go to the Setup workspace, and click the Status tab of the Instrument frame.

For more information on the Status tab of the Instrument frame, see Status Tab on page 66. See page 337 for troubleshooting information.

If you use the default settings and follow the recommended protocol, you can run a full 96-well plate in approximately 35 minutes.

Unloading the Plate

1 Once acquisition is complete, click the Unload button in the Plate Control frame to eject the plate from the plate sampler.

The Load and Unload buttons are disabled during acquisition.

2 Remove the plate from the plate holder.

Saving the Template

If you had to reposition the P1 gate to enclose the single population, save the template to save the new gate position. Reuse the template when running the same assay.

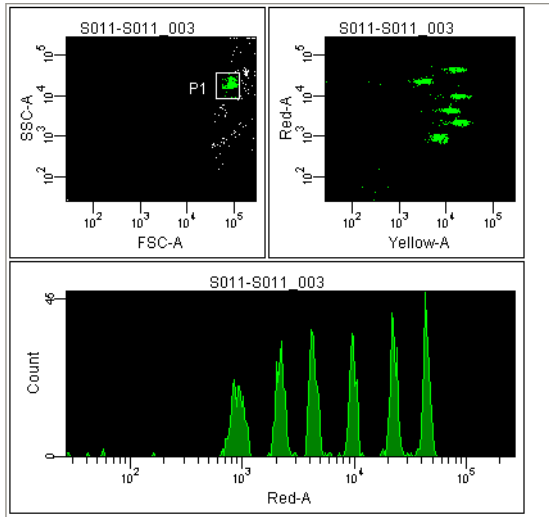
Reviewing the Plots

Review the plots to verify that the CBA data is acceptable. The following figures show an example of typical (Figure 6-8), acceptable (Figure 6-9 on page 190), and unacceptable (Figure 6-10 on page 191) CBA results.

Typical CBA Results

Examine the Yellow vs Red dot plot and the Red histogram. As shown in Figure 6-8, the populations on the dot plot should be clearly defined and tightly clustered. The peaks on the histogram should be single, well-separated peaks.

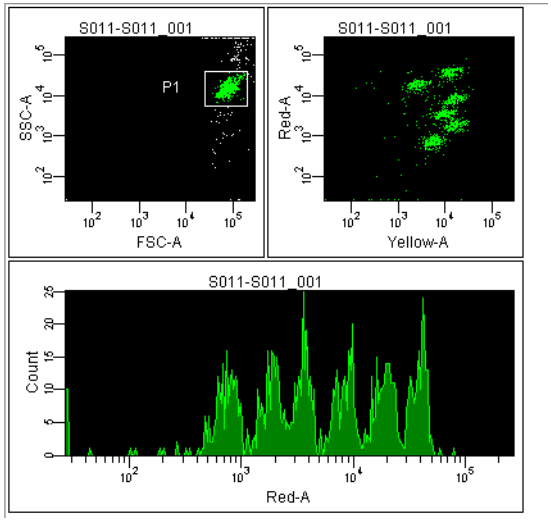
Figure 6-8 Typical CBA results



Acceptable CBA Results

Examine the Yellow vs Red dot plot and the Red histogram in Figure 6-9. The populations in the dot plot are not as clearly defined and tightly clustered as the typical data shown in Figure 6-8 on page 189. The peaks in the histogram are not as sharply separated as the typical data, but are distinct enough that the BD CBA software can still discern separate peaks.

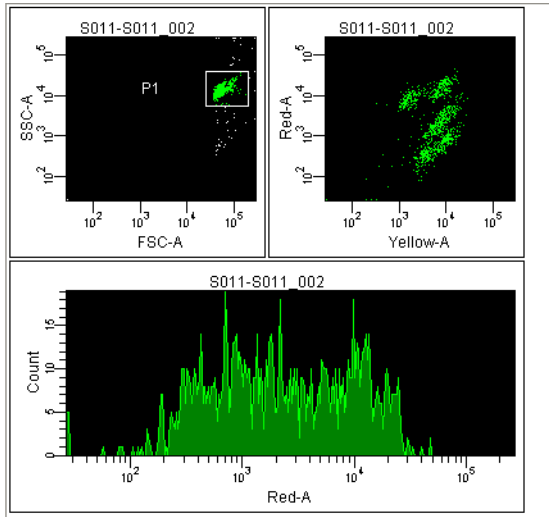
Figure 6-9 Acceptable CBA results



Unacceptable CBA Results

Examine the Yellow vs Red dot plot and the Red histogram in Figure 6-10. The populations in the dot plot are smeared, the peaks in the histogram are not distinct, and six separate populations cannot be discerned. This data could not be analyzed by BD CBA software.

Figure 6-10 Unacceptable CBA results



Reacquiring Samples

You can rerun samples if necessary, but the original data file will be overwritten. When rerunning samples, keep in mind your total sample volume, 20 µL overhead volume, and the plate-specific dead volume. See Understanding Volumes on page 105 for more information. Information on dead volume for plates compatible for use with the BD FACSArray bioanalyzer can be found in the BD FACSArray Plate Definition Guide packaged with your user's guide.

Saving the Plate for Reanalysis

1. After acquisition, store the covered plate at 4°C in case you want to reanalyze a sample.
2. Before reacquisition, vacuum dry the plate.
3. Resuspend the beads with 120 µL wash buffer.
4. Place the plate on a shaker for 2 minutes prior to acquisition.
5. Immediately analyze the plate on the bioanalyzer.

   The plate contains sufficient beads for at least two more sample acquisitions.

Analyzing BD CBA Data

Once you have acquired samples, export the CBA data in preparation for analysis with BD CBA software.

See Exporting BD CBA Data on page 284 for instructions on how to export the data.

Refer to the instructions included with the BD CBA assay kit or the *BD CBA Software User's Guide* for instructions on analyzing CBA data.

Running Multiple CBA Assays on One Plate

You can run multiple CBA assays on one plate, if necessary. Use the following tutorial to create an experiment with the Experiment Wizard to run two sets of CBA assays.

Creating the Experiment

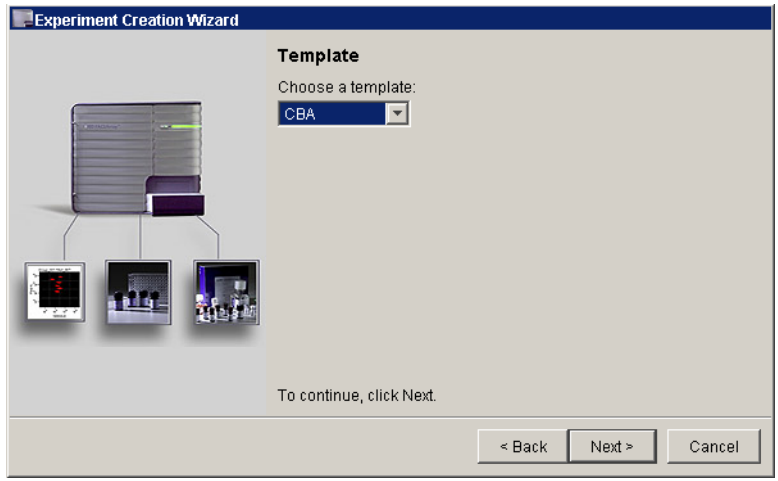
⚠ **NOTICE** Be sure that your sample layout on the plate matches what you set up using the Experiment Wizard. Sample layout created by the Experiment Wizard cannot be rearranged to match a pre-loaded plate.

1. Click the Experiment Wizard tool ( ) in the application toolbar.

   The Experiment Wizard Welcome dialog appears. This view remains for approximately 2 seconds and then automatically advances to the next view.

2. Choose the Default experiment description; click Next.

3. In the Template view, click the template drop-down menu and select CBA if not already selected; click Next.

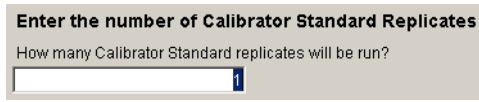


Entering Standard and Sample Information

1 Answer the series of questions concerning standards for the first assay only.

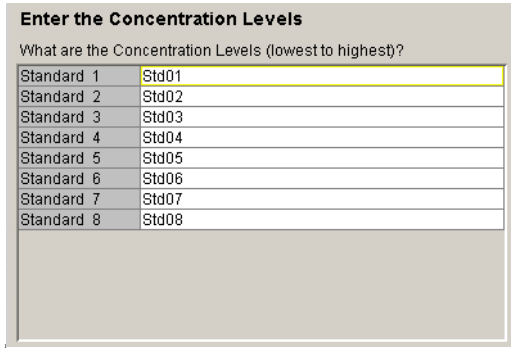
For this example, enter the following information in the corresponding views.

- In the Number of Calibrator Standard Replicates view, use the default of 1 replicate; click Next.



- In the Number of Concentration Levels view, use the default of 8 levels; click Next.

- In the Enter the Concentration Levels view, use the default concentration level names; click Next.

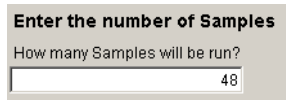


2 Answer the series of questions concerning samples for all assays combined.

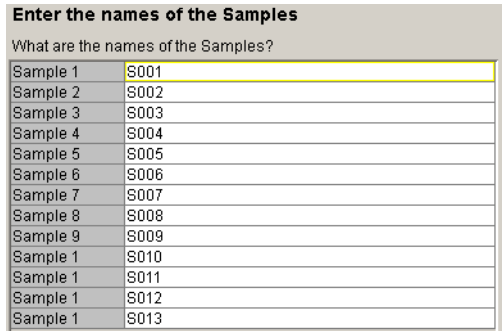
For this example, assay 1 contains 24 samples, and assay 2 contains 8 standards and 16 samples, for a total of 48 samples.

Enter the following information in the corresponding views.

- In the Number of Samples view, enter 48 for the number of samples to be run; Click Next.



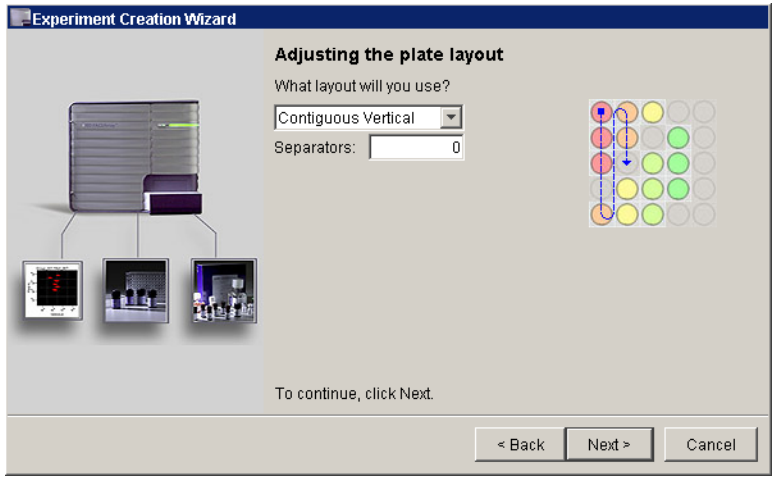
- In the Sample Names view, use the default names; click Next.



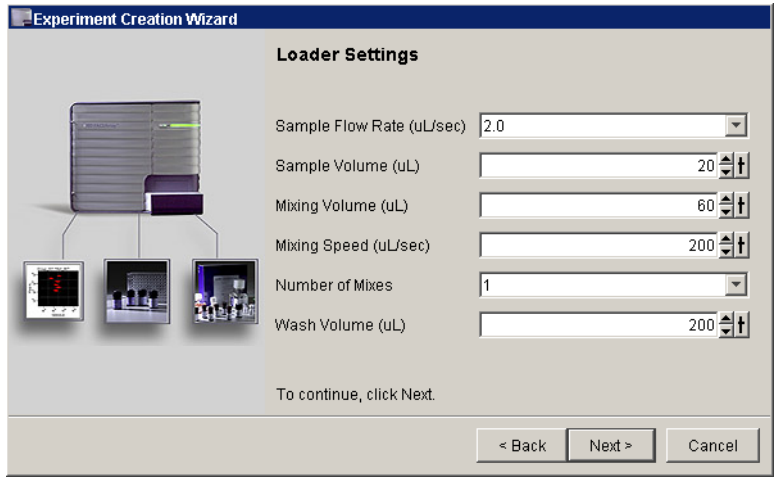
- Use the default setting of 1 for the number of sample replicates; click Next.

Choosing Additional Experiment Criteria

1 Choose the default settings of Contiguous Vertical with 0 Separators (blank wells to add between samples); click Next.



2 Use the default loader settings; click Next.

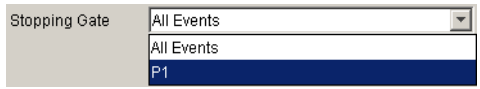


3 In the Acquisition Settings view, choose the number of events to acquire and the stopping gate.

For this example, choose the following options if they are not already selected.

- Click in the Events to Acquire drop-down menu, enter 1200 evt, and then press the Enter key on your keyboard.

- Click the Stopping Gate drop-down menu and choose P1 as the stopping gate.



Acquisition will stop when there are 1200 events in P1.

In the BD CBA template, an acquisition gate has been predefined. You will use the predefined gate (P1) as a stopping gate. A stopping gate triggers acquisition to stop when the specified number of events chosen in the Events to Acquire field has been reached.

NOTICE The default number of events is set to 1200 and is recommended for 6-bead assays such as Human TH1/TH2 Cytokine assay.

☑ **Tip** If you are running a single bead assay, adjust the Events to Acquire to 200.

4 Click Next.

Naming the Experiment and Wizard Session

1 In the Experiment Name view, enter a name for the experiment.

Name the experiment so that you will remember it is a single plate containing two different assays.

2 If you want to save the Wizard Session, click the Yes radio button and enter an appropriate name in the Saving Wizard Session view; click Next.

NOTICE If you prefer, you can use the same name for the Wizard session as you did for the experiment.

Reviewing the Wizard Session

1 Review the choices you have made in the Experiment Wizard summary view.

Use the scroll bar to see the entire view.

If you want to change any of the options you chose, click the Back button until you reach the appropriate page, make the change, and then click the Next button until you reach the summary page.

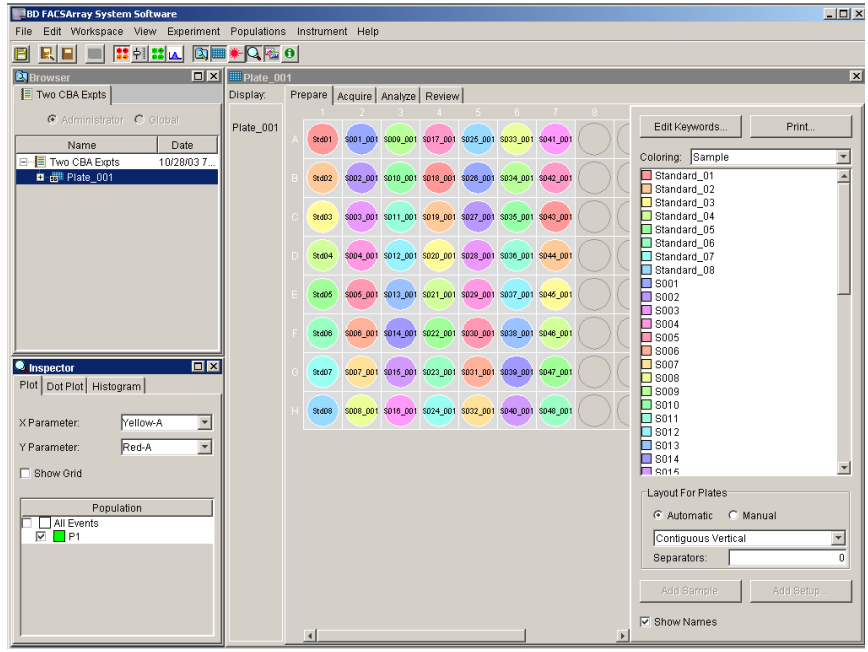
☑ **Tip** Print the information in the summary view by selecting the text in the summary box, pressing Ctrl-C on your computer keyboard, pasting the information into Microsoft Word or Wordpad, and printing the document.

2 Click Finish.

The experiment you just created opens in the Prepare workspace (Figure 6-11 on page 199).

- Standards for the first assay appear in the first column.
- Samples for the first assay appear in columns 2 through 4.
- Standards for the second assay appear in the fifth column.
- Samples for the second assay appear in columns 6 and 7.

Figure 6-11 Prepare workspace showing newly created experiment



3 If you prefer, you can rename the standards of the second assay.

- Click on well A5.
- In the Well Inspector, enter a new name for well A5.

  In this example, well A5 contains Std01.
- Press Enter on your computer keyboard to save the new name.

Analyzing the Data

Once you have saved data for all wells, you can export the data to analyze in BD CBA software. You will need to create extra folders to accommodate the standards and samples of the second assay.

1. Choose File > Export > CBA.

   Data is automatically exported into two folders, one folder contains data for the first set of standards, and the other contains the rest of the data.

2. Create two new folders with the following names.

   - CBA STANDARDS 2
   - CBA SAMPLES 2

3. Move the data for the second set of standards from the CBA SAMPLES folder to the new CBA STANDARDS 2 folder.

4. Move the data for the second set of samples from the CBA SAMPLES folder to the new CBA SAMPLES 2 folder.

5. Refer to the *BD CBA Software User's Guide* for details about analyzing exported CBA data.

7

Cellular Analysis

This chapter will guide you through experiment setup, acquisition, and analysis of two different types of cellular assays. The first experiment, an example of surface immunophenotyping using lysed whole blood, will be created with the Experiment Wizard. The other experiment, an example of viability testing of a cell line, will be created manually. A tutorial demonstrating advanced gating features follows, at the end of the chapter.

The following topics are covered in this chapter:

- Workflow Overview on page 203
- Four-Color Immunophenotyping Experiment on page 203
- Viability Testing Experiment on page 238
- Advanced Gating Features on page 258
- Running Multiple Assays on One Plate on page 268

Cellular samples from a variety of primary and secondary sources, for example, whole blood and cell lines, can be processed on the BD FACSArray bioanalyzer. The bioanalyzer measures characteristics such as forward scatter, side scatter, and up to four fluorescent colors of individual cells. Because antibodies conjugated to fluorophores bind specifically to cell surface antigens and intracellular proteins, different antibody combinations can be used to detect specific cell types.

Cellular applications compatible with the bioanalyzer include:

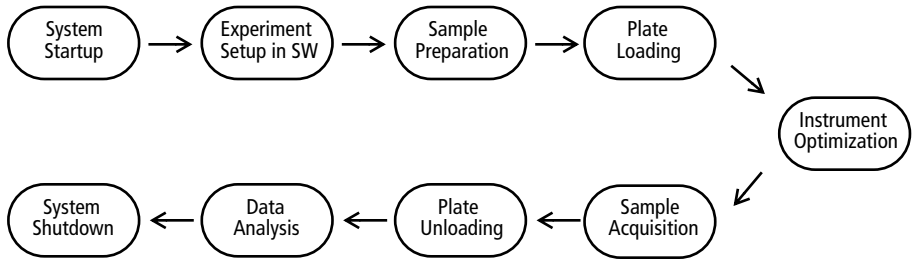
- Surface immunophenotyping (lymphocyte subsetting, surface Ig expression, immunotoxicology
- Live/dead discrimination (viability testing, apoptosis markers)
- Functional studies (cytokine detection, proliferation with BrdU, cell-signaling events, activation markers)
- Fluorescent protein analysis

Use the experiment examples that follow to help you create and customize experiments for your specific applications.

Workflow Overview

Figure 7-1 shows you an overview of the steps required to process and run samples on the BD FACSArray bioanalyzer. Use this overview to help you structure workflow for your applications.

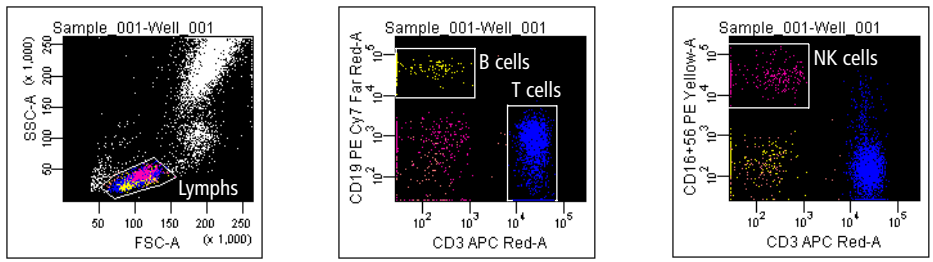
Figure 7-1 Workflow overview



Four-Color Immunophenotyping Experiment

Immunophenotyping is a method for characterizing cells based on the binding of fluorescently labeled antibodies. Up to four different antibody types, each bound to a different fluorochrome, can be combined to determine subpopulations of a particular cellular population. For example, by combining CD3 APC, CD16+CD56 PE, CD45 APC-Cy7, and CD19 PE-Cy7 in one well, you can determine the T-cell population ($CD3^+$), the B-cell population ($CD19^+$), and the NK-cell population ($CD16+CD56^+$).

Figure 7-2 Lymphocyte analysis showing B, T, and NK cells



Creating an Experiment Using the Wizard

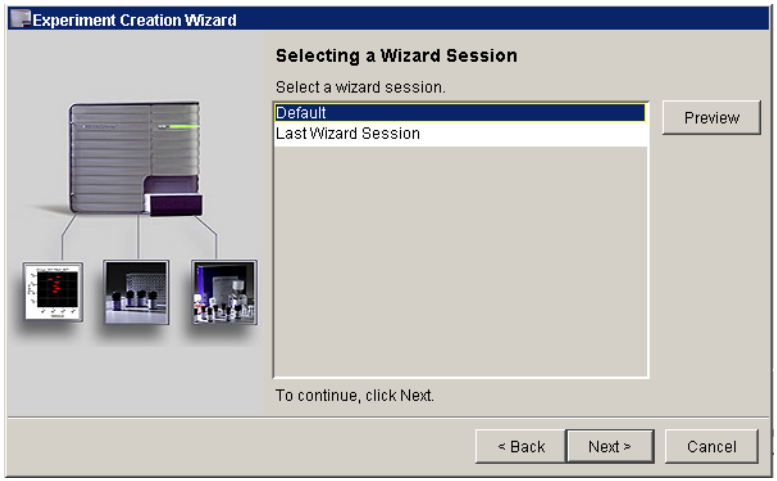
The following example shows you how to create a 4-color immunophenotyping experiment with human lysed whole blood using the Experiment Wizard.

Choosing a Wizard Session

1 Click the Experiment Wizard tool in the application toolbar.

The Experiment Wizard Welcome dialog appears. This view remains for approximately 2 seconds and then automatically advances to the next view (Figure 7-3).

Figure 7-3 Selecting a Wizard Session view



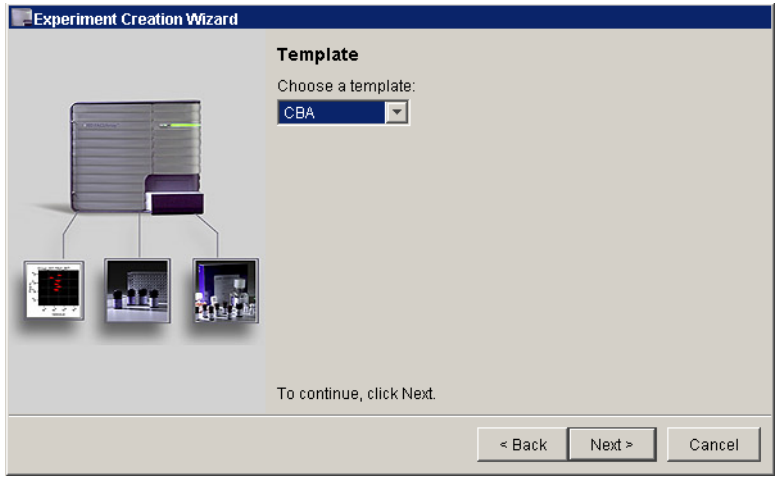
To view a summary of the selected wizard session, click the Preview button.

If you want to quit the Experiment Wizard session without creating the experiment, click the Cancel button on any of the Wizard views.

2 Choose the Default Wizard session; click Next.

The Template view appears (Figure 7-4) where you can choose a predefined template for the following types of experiments: cellular 1-, 2-, 3-, 4-, and multi-color; CBA; and QC. The templates contain default loader and instrument settings that serve as a starting point for sample setup and optimization for immunophenotyping and CBA applications.

Figure 7-4 Template view

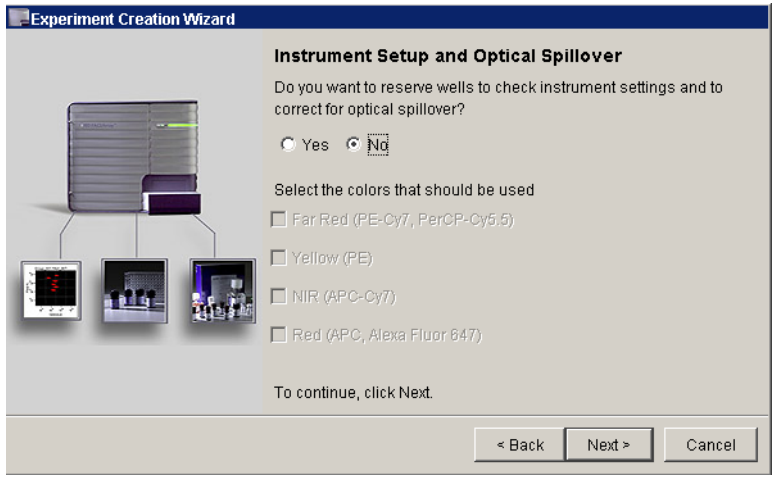


3 Choose the 4 Color template from the Template drop-down menu.

Entering Sample Information

1 Click Next.

The Instrument Setup and Optical Spillover view appears. For cellular experiments, you must add setup wells if you want the software to automatically calculate optical spillover. For cellular experiments using multiple colors that might require optical spillover correction, add a setup well for each color in your experiment. For more information about optical spillover, see Optical Spillover on page 96.

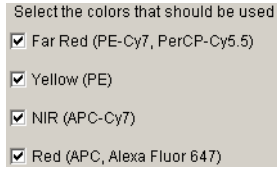

Experiment Creation Wizard
Instrument Setup and Optical Spillover
Do you want to reserve wells to check instrument settings and to correct for optical spillover?
Yes
No
Select the colors that should be used
Far Red (PE-Cy7, PerCP-Cy5.5)
Yellow (PE)
NIR (APC-Cy7)
Red (APC, Alexa Fluor 647)
To continue, click Next.
< Back
Next >
Cancel

2 Reserve setup wells.

For this example, reserve a setup well for each color.

- Click the Yes radio button (Yes) to enable the color selection checkboxes.
- Click in each checkbox to select all four colors (Figure 7-5 on page 207).

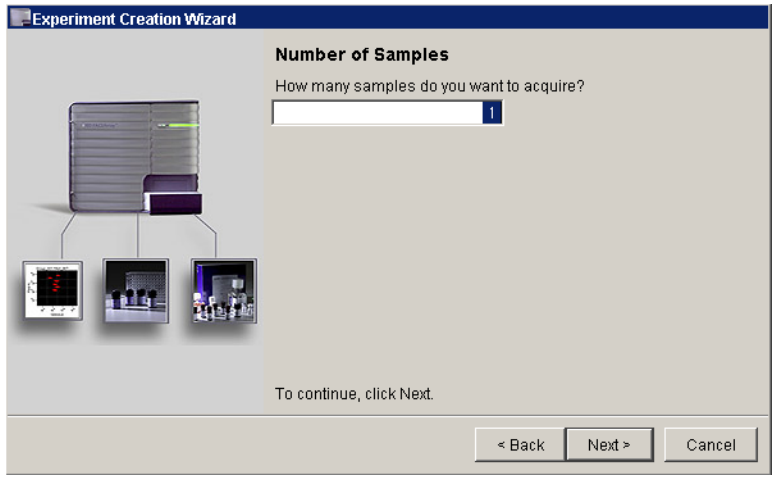
Figure 7-5 Color selection checkboxes



⚠ **NOTICE** When you choose to add setup wells to your experiment, an unstained well is also added. The unstained well is run before the other setup wells. For this example, a total of five setup wells are added.

3 Click Next.

The Number of Samples view appears.

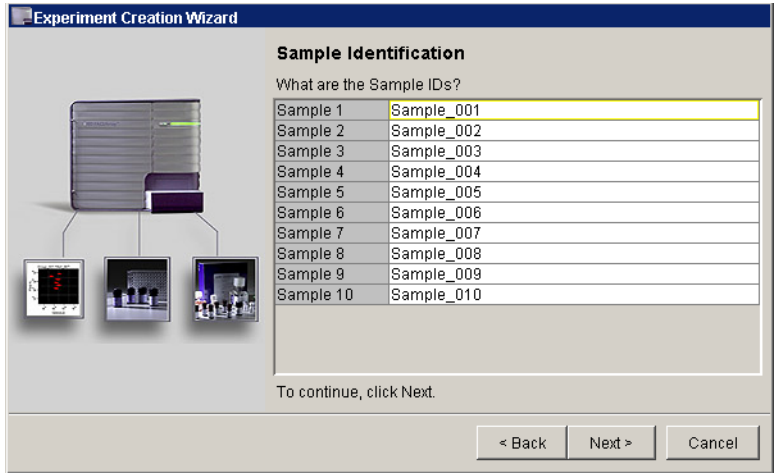


4 Enter the number of samples to acquire; click Next.

For this example, enter the number 10.

The Sample Identification view appears (Figure 7-6 on page 208).

Figure 7-6 Sample Identification view



5 Use the default sample identification for each sample.

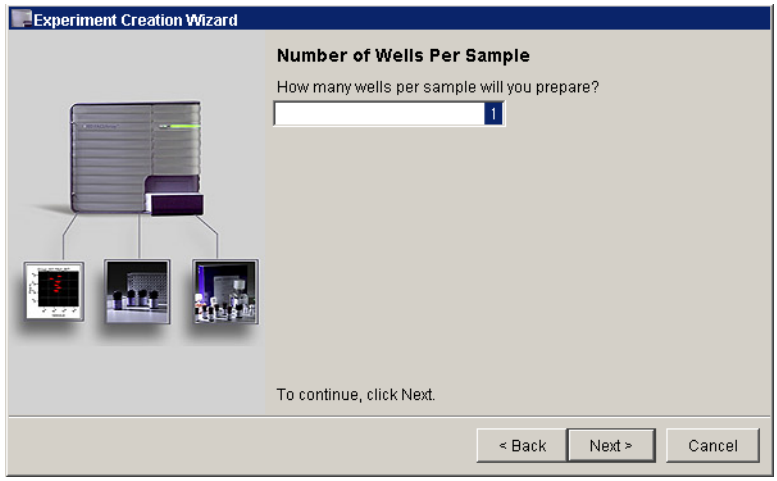
⚠ **NOTICE** To ensure that sample names can be saved by the software, do not use the following ASCII characters when specifying sample names: \, /, :, ?, “, <, >, ., or !.

Entering Well Information

1 Click Next.

The Number of Wells per Sample view appears (Figure 7-7 on page 209).

Figure 7-7 Number of Wells Per Sample view

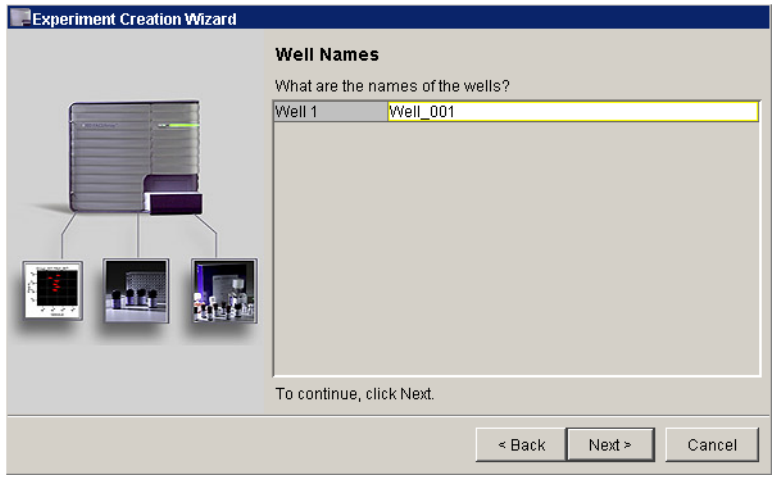


2 Enter the number of wells per sample to run, and then press Enter on your computer keyboard.

For this example, use the default of 1 well per sample.

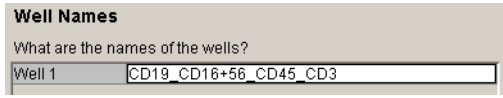
3 Click Next.

The Well Names view appears.



4 Enter the name of each well.

In this example, Well 1 contains reagents to identify T cells, B cells, and NK cells, so name well 1 *CD19_CD16+56_CD45_CD3*.



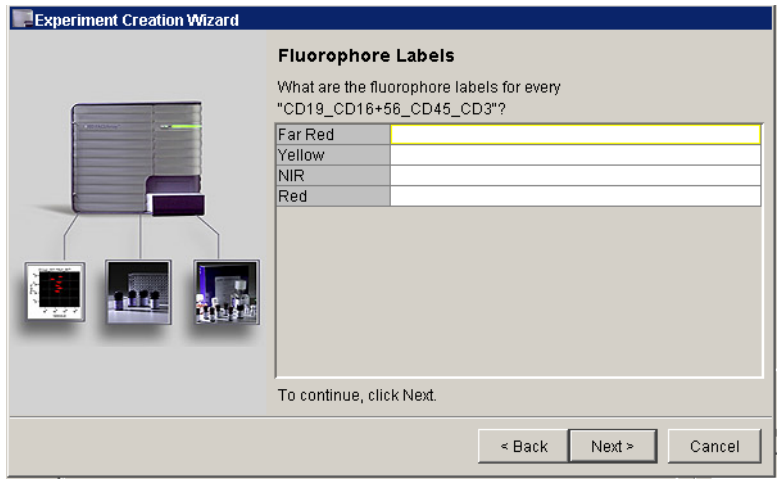
To enter text in the text field:

- Double-click in the white area of the text field to highlight the existing entry.
- Enter the new name.
- Press the Enter key on your computer keyboard

☑ **Tip** Click the tab button to easily move between text fields.

5 Click Next.

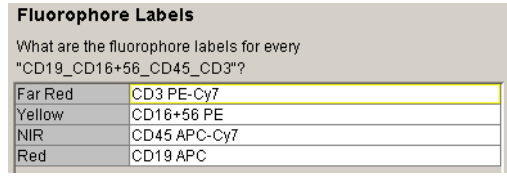
The Fluorophore Labels view appears. The fluorophore labels indicate the colors shown in each plot. The labels will appear as axes labels in plots displayed in the Template view in the Setup and Acquire workspaces.



6 Enter the name of each fluorophore, and then press Enter on your computer keyboard.

For this example, the fluorophores in well 1 are

- CD19 PE-Cy7
- CD16+56 PE
- CD45 APC-Cy7
- CD3 APC

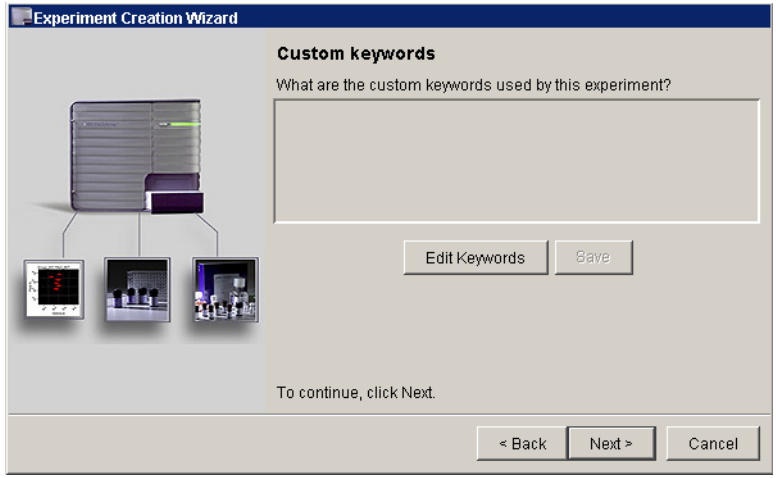


Choosing Additional Options

1 Click Next.

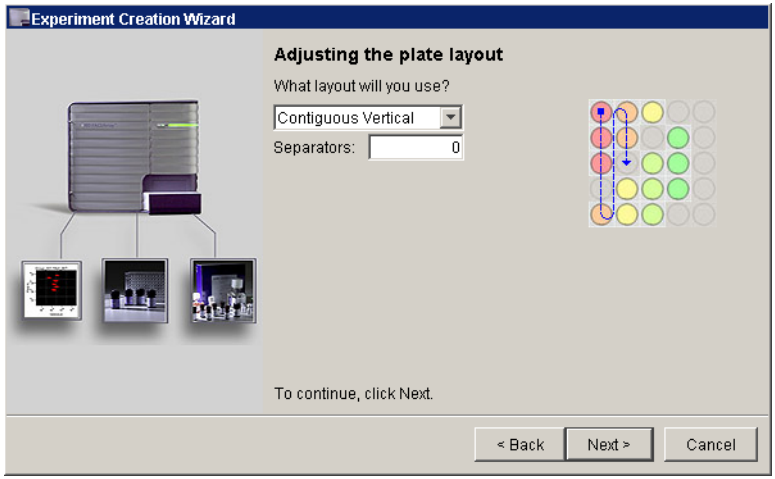
The Custom Keywords view appears (Figure 7-8). Do not add keywords in this Wizard session. For instructions on creating and editing keywords, see Adding Keywords on page 245.

Figure 7-8 Custom Keywords view



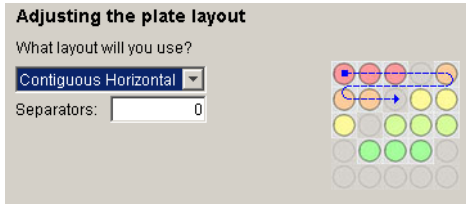
2 Click Next.

The Adjusting Plate Layout view appears. In this view, you can choose how to lay out samples on the plate. See Layout for Plates Control Group on page 74 for more information on the different plate layout options available.



3 For this example, choose Contiguous Horizontal from the drop-down menu, and 0 as the number of Separators; press Enter on your computer keyboard.

Separators are blank wells you can add between samples.

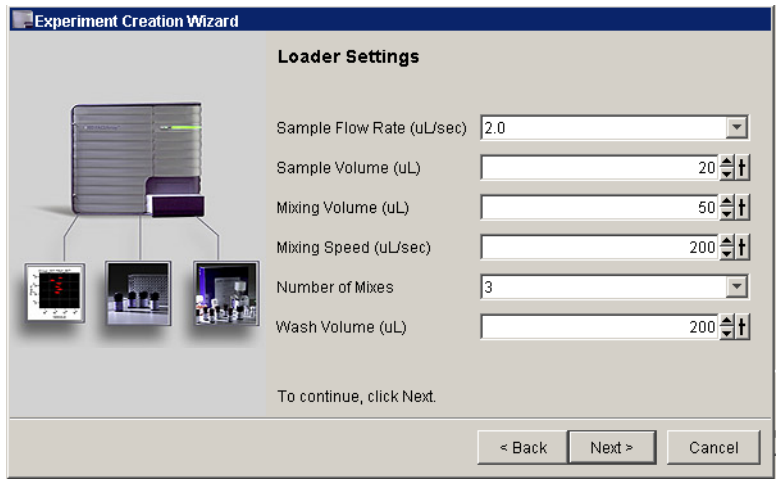


Specifying Loader and Acquisition Settings

1 Click Next.

The Loader Settings view appears.

Figure 7-9 Loader Settings view



⚠ **NOTICE** With the exception of sample flow rate, sample volume, and mixing volume, BD recommends using the default loader settings provided when using a BD template to set up your experiment. Change loader settings only for specialty applications and validate the new settings prior to routine use.

- When deciding on an appropriate sample volume, keep the following factors in mind.
  - The bioanalyzer aspirates the specified sample volume plus an additional 20 µL from each well.

    For example, if you specify a sample volume of 20 µL, a total of 40 µL of sample will be aspirated from each well.

- The sample volume you specify should be based on the cell concentration of the sample and the number of events you want to acquire.

  For example, if your sample concentration is 1,000,000 cells/mL (1,000 cells/µL) and you want to acquire 10,000 events, your sample volume should be at least 10 µL. Specifying a sample volume of 20 µL would give you plenty of leeway to acquire 10,000 events.

- The sample volume available for aspiration is affected by the plate-dependent dead volume. Information on dead volume for plates compatible for use with the BD FACSArray bioanalyzer can be found in the BD FACSArray Plate Definition Guide packaged with your user's guide.

• When deciding on an appropriate mixing volume, BD Biosciences recommends using a volume half to three-quarters the available volume. This ensures proper mixing of the sample without introducing air bubbles.

NOTICE Available volume = pipetted volume – dead volume. See Understanding Volumes on page 105 for more information.

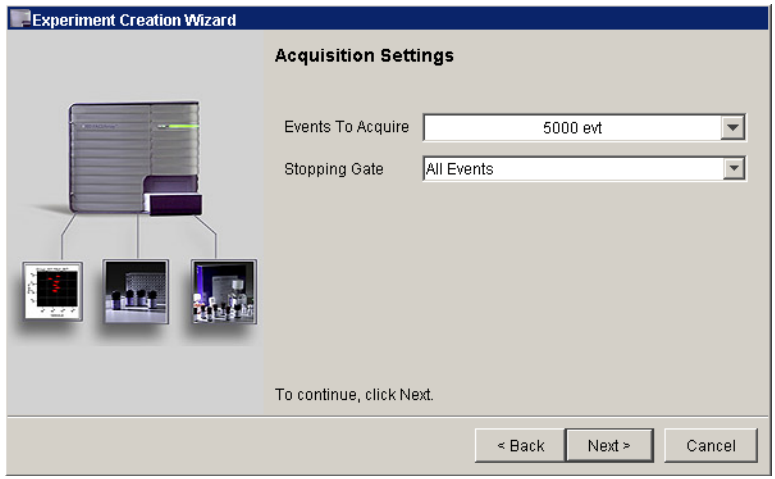
For information about other loader settings, see Inspector on page 103. For more information on sample, mixing, and dead volume, see Understanding Volumes on page 105.

2 Choose or enter loader settings, and then press Enter on your computer keyboard.

For this example, use the default loader settings. The default settings for this 4-color template are provided as a starting point for optimizing human lysed whole blood stained for 4-color immunophenotyping.

3 Click Next.

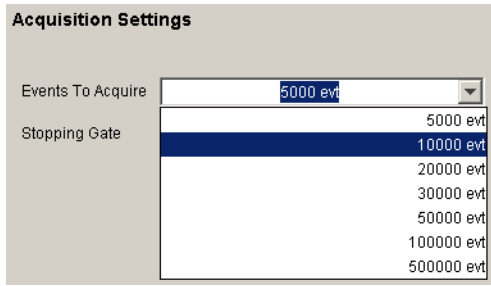
The Acquisition Settings view appears where you can choose how many events per well to acquire and whether to use a stopping gate.



4 Choose the acquisition settings.

For this example, choose the following settings.

- Click the arrow next to the Events To Acquire field and choose 10,000 events from the drop-down menu.



⚠ **NOTICE** If there are not enough cells to reach the specified number of events to acquire, acquisition will stop when the sample volume has been exhausted. You can adjust sample volume in the Loader Settings view (Figure 7-9 on page 213).

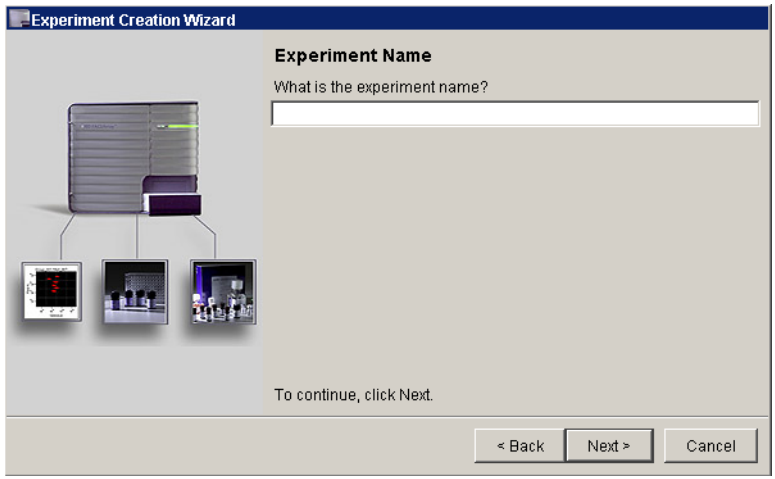
- Choose All Events as the Stopping Gate.

In this template, gates have not been predefined, so All Events is the only option available. If gates were predefined, each gate would appear in the Stopping Gate drop-down menu.

A stopping gate triggers acquisition to stop when the specified number of events chosen in the Events to Acquire field has been collected in the gate. You can choose any predefined gate as a stopping gate. For this example, a stopping gate is not defined.

5 Click next.

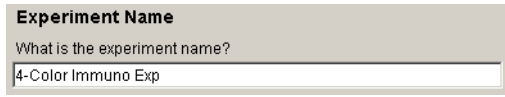
The Experiment Name view appears.



6 Enter a name for your experiment, and then press Enter on your computer keyboard.

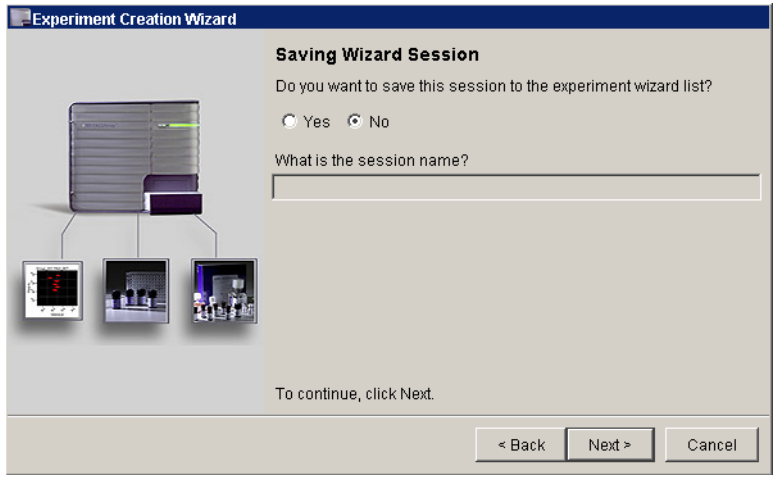
Use a name that will help you identify the experiment for future use. For example, you can name experiments according to the nature of the analysis to be performed, such as *CBA* or *Immunophenotyping*.

For this example, name the experiment *4-Color Immuno Exp*.



7 Click Next.

The Saving Wizard Session view appears where you can choose to name and save the Wizard session. Saving the Wizard session allows you to reuse the session for other similar experiments, but allows you to modify any of the existing settings.



8 Click the Yes radio button and enter the name of the Wizard session.

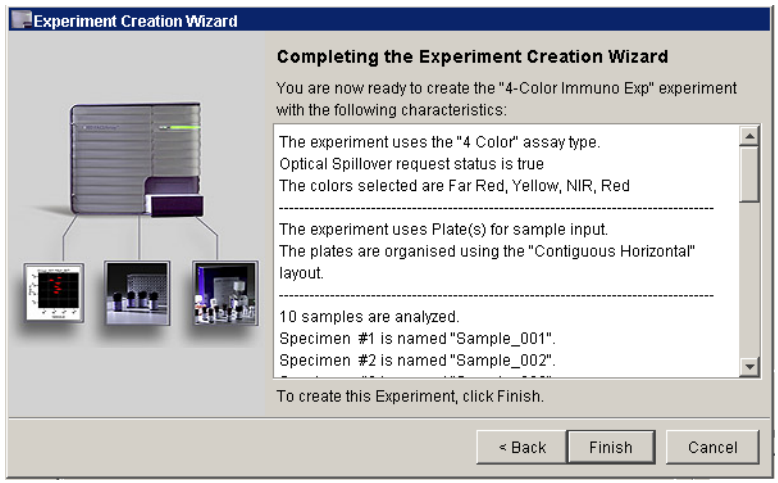
For this example, name the Wizard session *4-Color Immuno Wizard*.

If needed, you can use the same name for the Wizard session as you did for the experiment.

Reviewing Experiment Parameters

1 Click Next.

The Completing the Experiment Wizard summary view appears.


Experiment Creation Wizard
Completing the Experiment Creation Wizard
You are now ready to create the "4-Color Immuno Exp" experiment with the following characteristics:
The experiment uses the "4 Color" assay type.
Optical Spillover request status is true
The colors selected are Far Red, Yellow, NIR, Red
The experiment uses Plate(s) for sample input.
The plates are organised using the "Contiguous Horizontal" layout.
10 samples are analyzed.
Specimen #1 is named "Sample_001".
Specimen #2 is named "Sample_002".
To create this Experiment, click Finish.
< Back
Finish
Cancel

2 Review the choices you made.

Use the scroll bar to see the entire view.

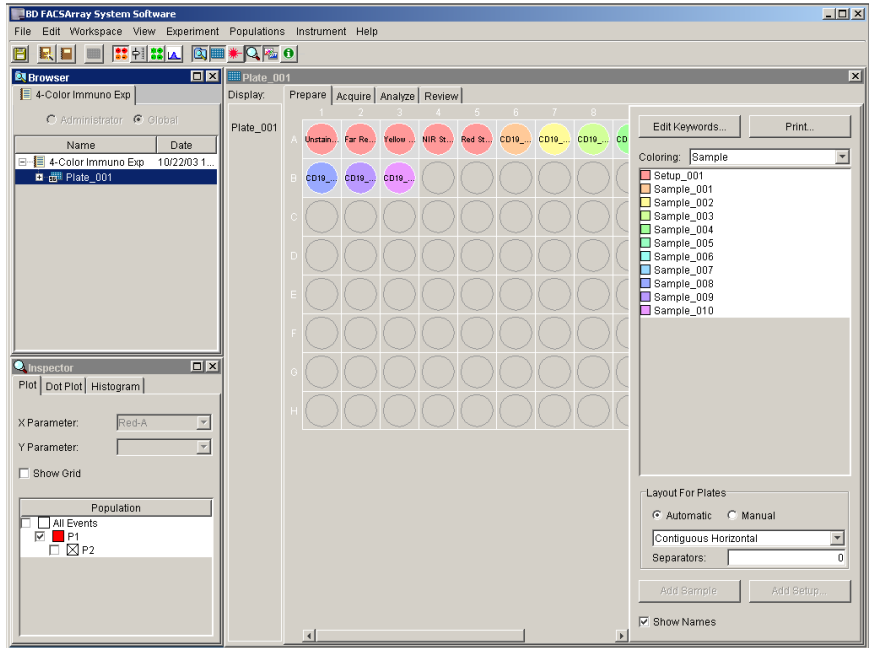
- If you want to change any of the options you chose, click the Back button until you reach the appropriate page, make the change, and then click the Next button until you reach the summary page.

- If you want to print the summary page, copy and paste the text into a word processing software.
  - Click the page and select all the text.
  - Use Ctrl-C to copy the text.
  - Paste the text into a word processing software program such as Microsoft Word.
  - Print the text.
- If do not want to save the experiment, click Cancel.

3 Click Finish.

The experiment you just created opens in the Prepare workspace.

Depending on the size of the experiment you just created, it might take several minutes for the Prepare workspace to appear.



4 In the Prepare workspace, review your plate layout.

Notice that the first five wells in the plate view have been designated as setup wells. These wells will be used to optimize instrument settings and for automatic optical spillover correction by the software.

5 If necessary, make additional modifications, such as adding keywords, changing acquisition settings, or adding samples. See Creating an Experiment Manually on page 238 for instructions.

6 Click well A1; change the sample flow rate and sample volume for the unstained well.

- Decrease the sample flow rate to 0.5 µL/sec (the minimum value).
- Increase the sample volume to 100 µL (the maximum value).

Decreasing the sample flow rate and increasing the sample volume for the setup wells allows you to view live events for a longer period of time while optimizing instrument settings.

7 Repeat step 6 for the remaining setup wells.

Preparing for Acquisition

WARNING All biological specimens and materials coming into contact with them can transmit potentially fatal disease. To prevent exposure to biohazardous agents, handle all biological specimens and materials as if capable of transmitting infection. Dispose of waste using proper precautions and in accordance with local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

NOTICE To avoid damaging the BD FACSArray bioanalyzer, do not run samples containing particles greater than 50 µm.

Preparing the Samples

You can either prepare your samples in tubes according to the appropriate reagent package insert, or develop and validate your own protocol for preparing samples directly in the 96-well plate.

1. For the Setup wells, prepare the appropriate amount of sample with the following single-color reagents.
    - Unstained sample
    - CD19 PE-Cy7
    - CD16+CD56 PE
    - CD45 APC-Cy7
    - CD3 APC
2. Using the reagents selected in the Wizard session, prepare the remaining samples for your experiment.

Calculating the Amount of Sample Needed for Optimization

Because you chose to add wells to check instrument settings and correct for optical spillover, you must decide how many microliters of sample you will need for optimization. The amount of sample needed for optimization depends on the following factors.

- Sample flow rate (selected in the Wizard session)
- Sample volume (selected in the Wizard session)

⚠ **NOTICE** The bioanalyzer will aspirate the selected sample volume plus an additional 20 µL from each well.

- Number of times sample is aspirated from the well

- Total volume loaded into the well should equal or exceed the plate-dependent dead volume plus the number of aspirations times the sum of the sample volume plus overhead volume (20 µL).

  total volume per well ≥ dead volume + number of well aspirations x (sample volume + 20 µL)

  For example, if you use a plate with a 30 µL dead volume and plan to sample twice from each well using the default sample volume for cellular analysis, each well should contain at least 110 µL of sample.

  30 µL + 2 x (20 µL + 20 µL) = 110 µL of sample

The first few times you run an experiment, BD recommends adding the maximum volume allowable to the setup wells because each setup well will be sampled at least twice while optimizing instrument settings. The maximum volume varies depending on plate type. Information on dead volume for plates compatible for use with the BD FACSArray bioanalyzer can be found in the BD FACSArray Plate Definition Guide packaged with your user's guide. Make sure you have extra sample in case you need to rerun the setup wells.

Preparing the Plate

☑ **Tip** Use a printout of the plate view as a guide for transferring prepared samples to the 96-well plate.

1. Print the plate view of the experiment by clicking the Print button in the upper right corner of the Prepare workspace.
2. Add the appropriate volume of prepared sample to the corresponding wells.
   - Unstained sample in well A1
   - PE-Cy7–stained sample in well A2
   - PE-stained sample in well A3
   - APC-Cy7–stained sample in well A4
   - APC-stained sample in well A5

3 Follow the plate view printout to transfer prepared samples to the 96-well plate.

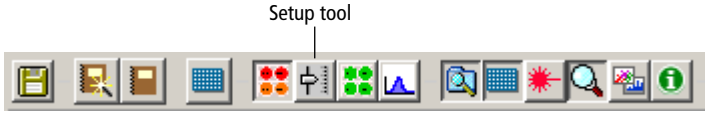
Loading the Plate

⚠ **CAUTION** Movement of mechanical parts within the instrument can pinch or injure your hands or fingers. To prevent injury by moving parts, follow these precautions.

- Keep your hands away from the plate holder once you position the plate on it. The plate holder will automatically retract when you click Load.
- Keep your hands away from the plate door during instrument operation.

⚠ **NOTICE** To prevent damage to the bioanalyzer, remove any lid, film, or cover from the plate before loading onto the plate holder. Some combinations of plate and lid might not retract properly into the instrument, potentially causing damage to the plate and the plate sampler. Leaving a lid, film, or cover on the plate might cause clogs in the probe and sample line, affecting performance and possibly damaging the bioanalyzer.

1 Click the Setup tool in the application toolbar to open the Setup workspace.



The wells you selected for acquisition appear in the plate view, and the setup wells are numbered in acquisition order.

2 Place the plate containing the stained samples on the plate holder.

Position the plate so that well A1 is over the A1 mark on the plate holder (Figure 7-10 on page 224).

Figure 7-10 A1 mark on plate holder

3 Click the Load button in the Plate Control group.

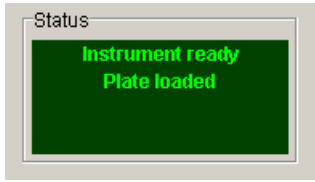
The plate will be retracted three-quarters of the way into the bioanalyzer.

- If a lid is detected on the plate, the plate will be ejected from the bioanalyzer, and the following message will appear on the computer monitor.

Remove the lid and click the Load button in the Plate Control frame to reload the plate.

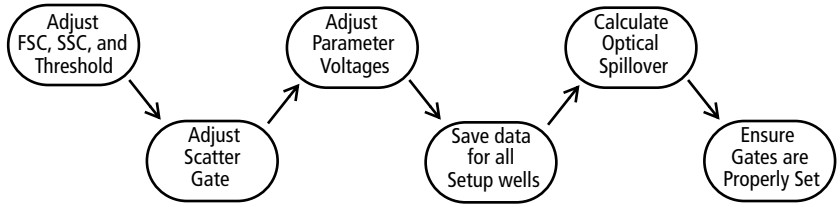
- If no lid is detected on the plate, the plate is completely retracted into the bioanalyzer.

  The Status box should display the following message.



Preparing for Optimization

Default instrument settings are provided for immunophenotyping of human blood cells prepared using a lyse/wash method. These settings should put this cell type on scale, but settings might need to be optimized for your sample type. The following section shows how to optimize settings for a 4-color assay. Optimization is a multistep process consisting of the following steps.


Adjust FSC, SSC, and Threshold
Adjust Scatter Gate
Adjust Parameter Voltages
Save data for all Setup wells
Calculate Optical Spillover
Ensure Gates are Properly Set

For simplicity, the values column in the Parameters tab of the Instrument frame is titled *Voltages*. To be specific, the SSC, Yellow, and Red parameters are detected with PMTs. Adjusting the voltage applied to PMTs alters the amplitude of the detected signal. The voltage setting in the Parameters tab is representative of the voltage applied to the PMTs.

However, FSC, Far Red, and NIR parameters are detected with solid-state detectors (photodiode for FSC, APDs [Avalanche photodiode] for Far Red and NIR). For these parameters, adjusting the amplifier gain alters the amplitude of the detected signal. The Voltage setting causes the amplifier gain to change in an approximately logarithmic fashion, similar to the PMT response for SSC, Yellow, and Red. For the solid-state detectors used for FSC, Far Red, and NIR, a Voltage setting of 1 results in the minimum amplifier gain.

When setting the Threshold, be careful to avoid setting it too close to the minimum expected value of your data. The threshold is a boundary below which data will not be acquired and is set on the unprocessed signal amplitude. The value of the processed data parameter (pulse area) is likely to be somewhat higher or lower than the unprocessed value used to set the threshold.

Before viewing live events, keep in mind the following information.

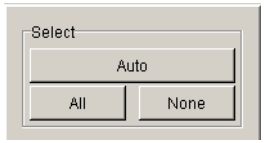
- In Setup mode, data is not saved. Events are displayed continuously until you stop acquisition or the sample volume aspirated is exhausted.
- When you click Acquire, the sample volume you specified in the Wizard will be aspirated and processed, and data will be saved.
- Once acquisition is in process, clicking Setup or Acquire to stop viewing live events will not return unused sample to the well.
- The bioanalyzer will aspirate the specified sample volume plus an additional 20 µL from each well.
- If you need to resample a well, verify you have enough available volume. See Understanding Volumes on page 105 for additional information.

Optimizing Instrument Settings

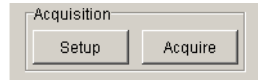
Optimizing FSC, SSC, and Threshold Settings

To perform this set of optimization steps, you need to view data only from the first well.

1. In the Select Control group, click None to deselect the wells, and then click well A1.



2. Click the Setup button in the Acquisition Control group.



   Events appear in the plots, but data is not being saved.

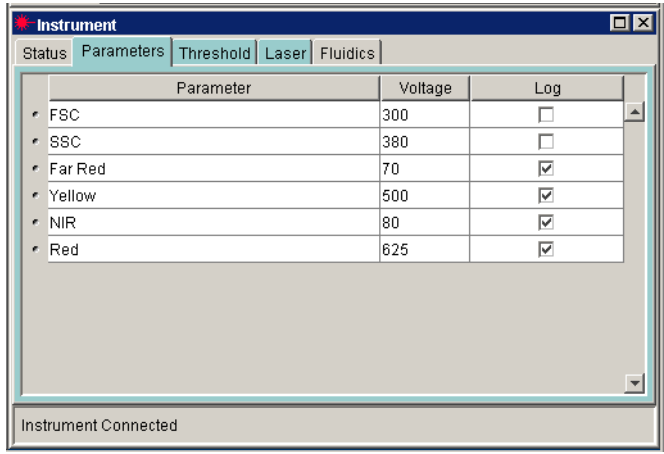
3. Adjust the FSC and SSC voltages to place the population of interest on scale in the FSC vs SSC plot.

   Adjust the signal for events displayed in plots by changing voltage settings. Higher voltage levels increase signal; lower voltage settings decrease signal.

   - Click the Parameters tab in the Instrument frame to display the Parameters pane (Figure 7-11 on page 228).
   - Click in the Voltage field to edit the setting. Voltages can be adjusted from 1–1,000.
   - Press Enter on your computer keyboard to save the changes.

     For more detailed information on parameters, see Parameters Tab on page 67.

Figure 7-11 Parameters tab of Instrument frame

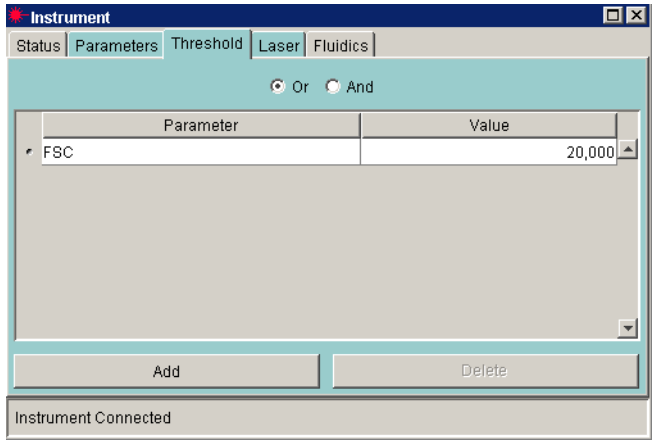


4 Adjust the threshold to eliminate noise.

- Click the Threshold tab in the Instrument frame to display the Threshold pane (Figure 7-12 on page 229).
- Click in the Value field to edit the setting. You can choose one or two threshold values.
- Press Enter on your computer keyboard to save the changes.

For more information about threshold, see Threshold Tab on page 68.

Figure 7-12 Threshold tab of Instrument frame



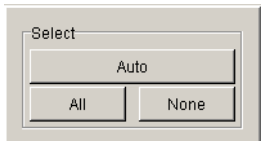
5 Click Setup to stop viewing live events.

Optimizing Fluorescence Settings

The goal in optimizing fluorescence settings is to ensure that the brightest stained cells do not appear off scale (greater than channel 262,143) while the dimly fluorescent cells remain on scale. BD recommends that you use the brightest sample of each fluorochrome in your experiment for each single-stained setup well.

For best sensitivity, the brightest and dimmest populations should be as well separated as possible while keeping both on scale. For best resolution of dim populations, keep the mean channel of dimly stained (not unstained) populations above channel 300.

1 In the Select Control group, click None to deselect the wells, and then click well A2.



2 Click the Setup button in the Acquisition Control group.

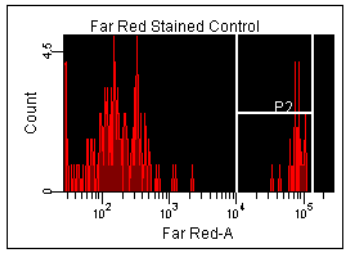
Events appear in the plots, but data is not being saved.

3 Adjust the gate in the dot plot to surround the lymphocytes.

NOTICE The histogram plot displays only gated events.

4 While observing the Far Red histogram, perform the following:

- Adjust the Far Red voltage if necessary to place the brightest population on scale.
- Verify the dim fluorescent cells remain on scale.



5 Click Setup to stop acquisition.

6 Repeat step 1 through step 5 with wells A3–A5 for the Yellow, NIR, and Red parameters while observing the corresponding plots.

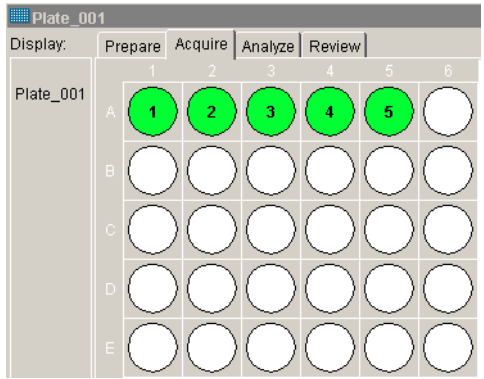
NOTICE After this step, each setup well will have been sampled at least once.

Acquiring Data to Calculate Optical Spillover

The last step is to save the setup well data so that the software can use the data to automatically calculate and apply optical spillover correction.

⚠ **NOTICE** Verify that you have enough available sample volume in wells A1–A5 to acquire and save data. If not, add more sample to the wells. For more information, see Understanding Volumes on page 105.

1 In the plate view, verify setup wells A1–A5 are selected.

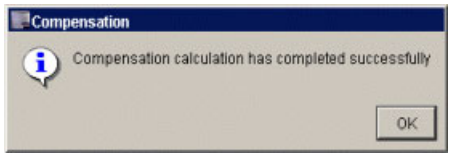


2 Click Acquire.

Data will be saved for all five wells. After the last setup well is saved, the software automatically calculates and applies optical spillover correction. It does this by

- Setting interval gates around the negative population in each fluorescence parameter based on data from the unstained well
- Setting gates around the positive population based on data from each single-stained well

If optical spillover correction was successfully calculated, the following message appears.



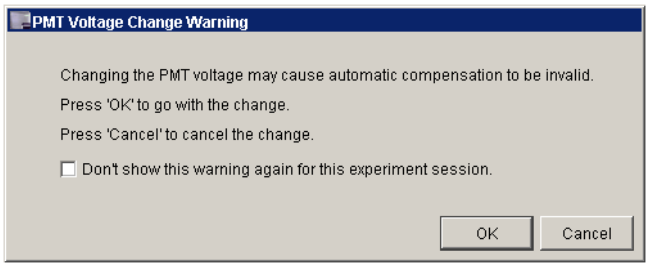
Ensuring Gates Were Properly Set

⚠ **NOTICE** If you adjust any of the gates in the section, you must recalculate optical spillover by choosing Instrument > Calculate Spillover.

1. Click the Analyze tab in the Plate Editor.
2. Click the first well in the plate view and then examine the plots in the Template view.
3. If needed, perform the following:
   - Click on the gate in the FSC vs SSC plot and drag it to the population you want to identify.
   - Adjust interval markers in each histogram to enclose the negative population.
4. Click in each of the remaining setup wells and verify:
   - the gate in the FSC vs SSC plot surrounds the population you want to identify.
   - interval gates enclose the positive population in each fluorescence parameter.

⚠ **NOTICE** If you adjust parameter settings in the Instrument frame after spillover has been calculated, the following message appears (Figure 7-13). You will need to rerun the setup wells and recalculate spillover.

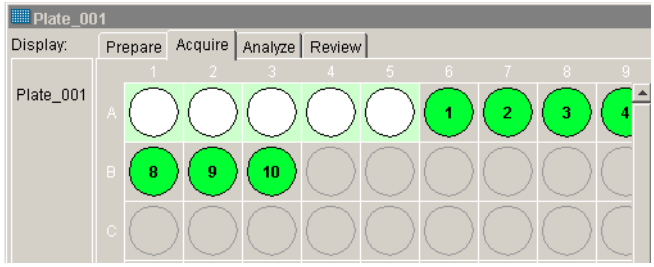
Figure 7-13 PMT Voltage Change Warning message


PMT Voltage Change Warning
Changing the PMT voltage may cause automatic compensation to be invalid.
Press 'OK' to go with the change.
Press 'Cancel' to cancel the change.
Don't show this warning again for this experiment session.
OK
Cancel

Acquiring Samples

1 In the Select Control group, click the Auto button.

The ten samples remain selected, and well A6 is numbered as one.



2 Click the Acquire tool ( ) in the application toolbar to open the Acquire workspace.

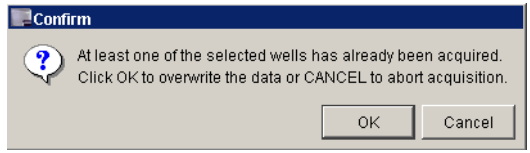
3 Click the Acquire button in the Acquisition Control group.

After a short pause, the following occurs.

- An orange ring appears in the first well selected for acquisition indicating that sample is being acquired.

- Events appear in the plots.
- Data is saved.

If any of the wells selected for acquisition already contain data, an alert dialog appears.


Confirm
At least one of the selected wells has already been acquired.
Click OK to overwrite the data or CANCEL to abort acquisition.
OK
Cancel

- Clicking OK will begin acquisition and existing data will be overwritten.

- Clicking Cancel will abort acquisition.

For instructions on how to reacquire a well without overwriting existing data, see Reacquiring a Well on page 238.

What to Monitor During Acquisition

1 Monitor the Status box for error messages.

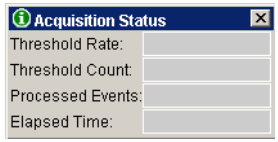
If an instrument error occurs during acquisition, a message will be displayed on the Status box in the Acquire workspace.

2 To view detailed error information, open the Instrument frame by clicking the Instrument tool ( ) in the application toolbar and click the Status tab.

For more information on the Status tab of the Instrument frame, see Status Tab on page 66. See page 337 for troubleshooting information.

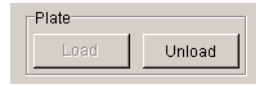
3 Click the Acquisition Status tool ( ) in the application workspace to open the Acquisition Status frame.

The Acquisition Status frame is a useful tool to monitor whether events are being detected and processed during acquisition.



Unloading the Plate

1 Once the plate has been acquired, click the Unload button in the Plate Control group to eject the plate from the plate sampler.



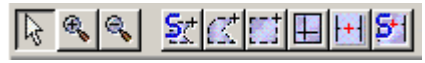
2 Remove the plate from the plate holder.

Data Analysis

Use the following instructions to analyze your data.

1 Click the Analyze tool in the application toolbar to open the Analysis workspace.

2 Use the template toolbar to set any gates for analysis.

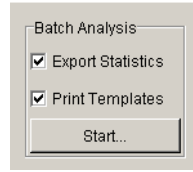


3 Review data for a few wells to ensure gates created encompass the appropriate population.

Because the same gates are applied to all wells in the experiment, this step ensures that gates created are appropriately set to analyze the data in most wells.

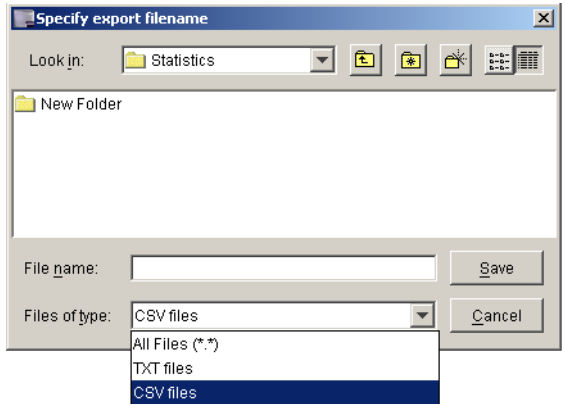
4 Choose to export statistics and print the templates by clicking the two radio buttons in the Batch Analysis Control group.

5 Click start.



The export dialog appears (Figure 7-14 on page 236).

Figure 7-14 Export dialog



- Use CSV files (comma separated values) for spreadsheet applications such as Microsoft Excel.

Any commas in the text of exported fields will cause the text to be split into two cells in the spreadsheet application.

- Use TXT files for wordprocessing applications such as Microsoft Word.

6 Click Save.

A progress bar appears. The data files will be analyzed using the predefined analysis gates.

Data Review

You can use the review feature to view data in a selected plot to do the following plate-based assessment of data.

- View images of data
- Quickly verify a Snap-To gate is enclosing the population of interest
- Screen for specific populations

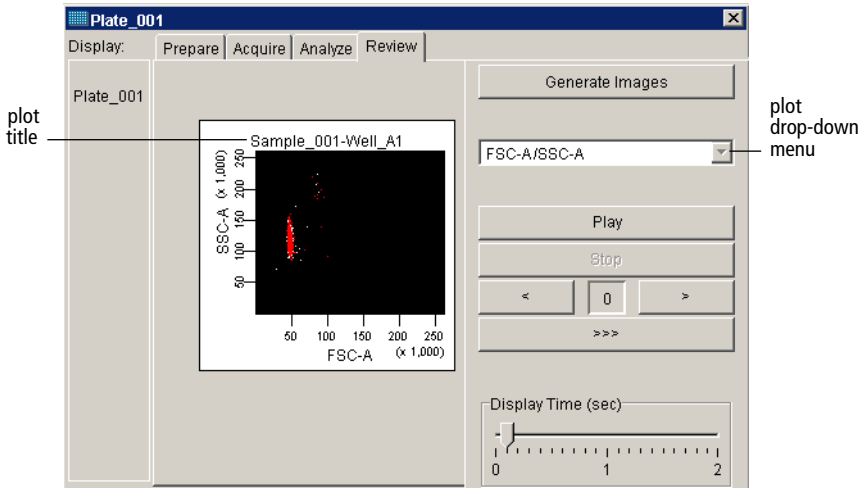
Use the following instructions to review the data analysis.

1 Click the Review tab in the Plate Editor.

2 Click the Generate images button in the Review Control group.

The software will quickly convert the results in the Template view to a series of images of each analyzed plot. Once the conversion is complete, the remaining buttons in the Review Control group are enabled.

NOTICE The plot title displays the sample and well name to identify the data.



3 Choose the plot to display from the drop-down menu.

4 Use the forward (>) and reverse (<) buttons to display plot images from different wells.

Alternatively, choose the Display Time, and then click Play to view plot images in succession.

Reacquiring a Well

If after reviewing the data, you want to reacquire a well without overwriting the data, perform the following steps.

1 Add a plate to the experiment in which the data was acquired.

2 Recreate the well with the same loader and acquisition settings.

3 Go to the Acquire workspace and acquire the well.

Viability Testing Experiment

Viability testing is a method for determining the percentage of live cells versus dead cells. In this example, you will set up a single-color experiment for analyzing eight Raji cell line samples stained with propidium iodide (PI). Use this tutorial as a guide to help you create and customize experiments for your specific applications.

Creating an Experiment Manually

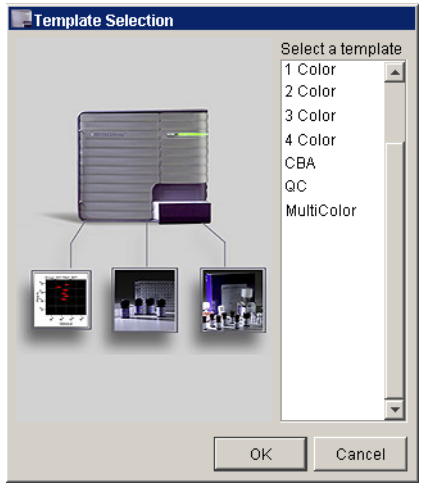
You can create experiments using the Experiment Wizard or you can create experiments manually. BD recommends using the Experiment Wizard when possible because it simplifies experiment setup. For the times you want to set up a simple experiment manually, use this tutorial as a guide.

Choosing an Experiment Template

1 Click the New Experiment tool in the application toolbar.

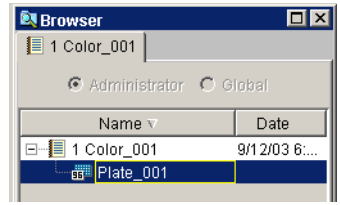
Alternatively, choose Experiment > New Experiment.

The Template Selection dialog appears.



2 Select the 1 Color template from the drop-down menu; click OK.

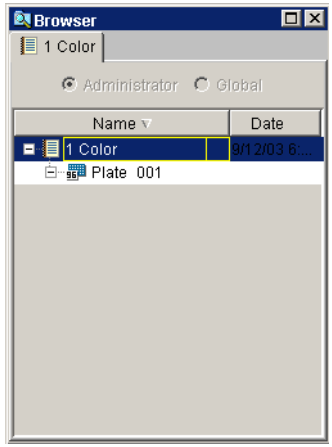
The 1 Color experiment appears in the Browser with Plate 001 selected.



Renaming the Experiment

You can modify the name of an experiment, plate, sample, or well using the Browser. You can also use the corresponding Inspector to edit the name.

1 In the Browser, click the experiment name (1 Color) to select it.



2 Click in the Name field.

1 Color becomes selected.

3 Enter the name *Viability;* press the Return key on your computer keyboard.

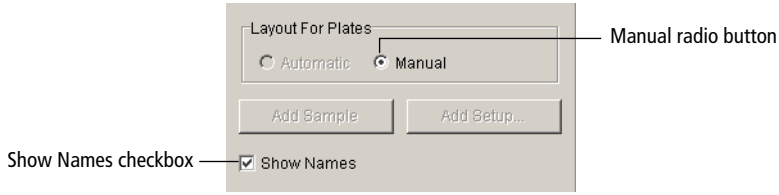
Adding Samples to the Experiment

1 Verify that the Prepare tab is selected in the Plate Editor.

2 In the Browser, click the plate name (Plate_001).

Notice that the Manual button in the Layout For Plates Control group is enabled (Figure 7-15 on page 241).

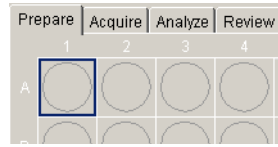
Figure 7-15 Layout For Plates Control group



3 If not already selected, click the Show Names checkbox to select it.

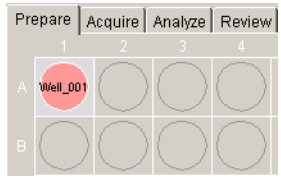
4 Click well A1 in the plate view.

A blue line appears around the well, and the Add Sample button in the Layout For Plates control group becomes enabled.

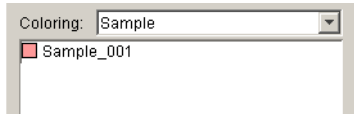


5 Click the Add Sample button.

In the plate view, well A1 turns color and the name *Well_001* appears in the well.



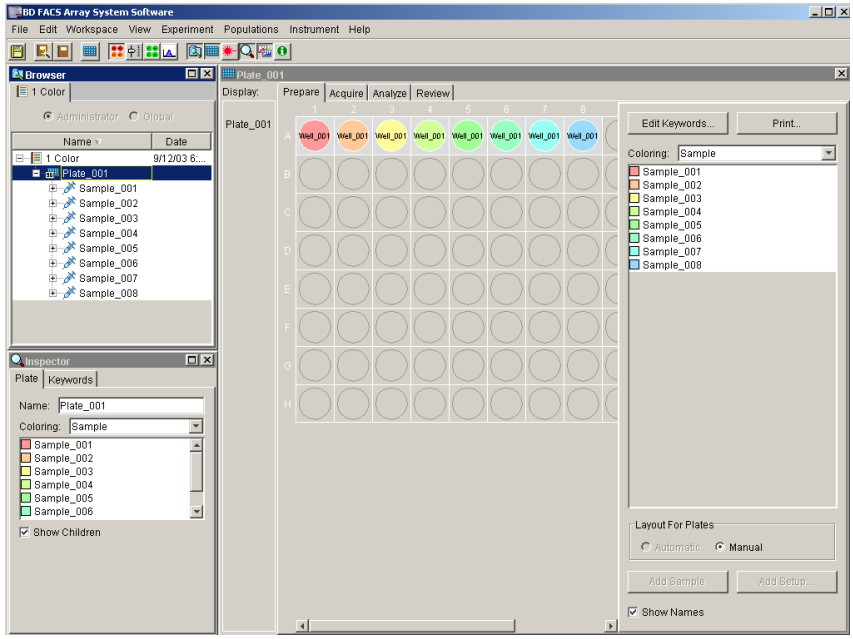
In the Coloring legend, *Sample_001* appears next to a colored box that corresponds to the new well color.



6 Repeat steps 4 and 5 to add seven more samples.

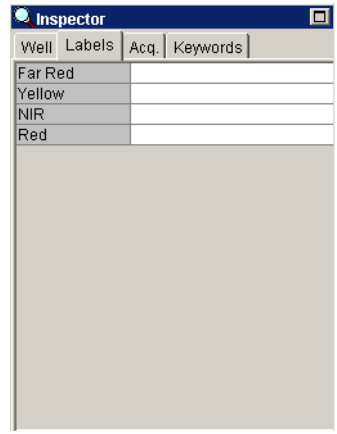
The Prepare workspace should look similar to the following figure (Figure 7-16 on page 242).

Figure 7-16 Prepare workspace



Editing Fluorophore Labels

1. Click well A1 in the plate view.
2. In the Well Inspector, click the Labels tab.
3. Click in the field next to the fluorophore to add a label.
4. For this example, enter PI in the Yellow field; press Enter on your computer keyboard.



NOTICE When in manual mode, you need to repeat step 1 through step 2 for each well.

Verifying Loader Settings

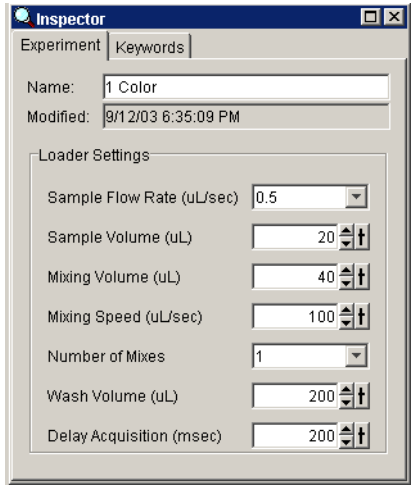
1 Click the open experiment icon ( ) in the Browser.

2 If necessary, click the Experiment tab in the Inspector (Figure 7-17).

3 View the default loader settings provided with the 1 Color template.

⚠ **NOTICE** With the exception of sample flow rate, sample volume, and mixing volume, BD recommends using the default loader settings provided when using a BD template to set up your experiment. Change loader settings only for specialty applications, and validate the new settings prior to routine use.

Figure 7-17 Experiment Inspector displaying Experiment tab



NOTICE Loader settings can also be set per well by clicking the well in the plate view, and then selecting the Acq. tab in the Well Inspector.

4 In the plate view, select well A1 and then select the Acq. tab in the Well Inspector.

5 Change the sample volume to the maximum amount allowable (100 µL), if needed.

The first few times you run an experiment, BD recommends adding the maximum volume allowable to the first well because you will use sample from the first well to optimize instrument settings. The maximum volume varies depending on plate type. Information on dead volume for plates compatible for use with the BD FACSArray bioanalyzer can be found in the BD FACSArray Plate Definition Guide. Make sure you have extra sample in case you need to rerun the setup wells.

6 Click the open experiment icon in the Browser.

7 If necessary, edit the sample flow rate, sample volume, and mixing volume for wells A2–A8.

When deciding on an appropriate sample volume, keep the following in mind.

- The bioanalyzer aspirates the specified sample volume plus an additional 20 µL.

  For example, if you specify a sample volume of 20 µL, a total of 40 µL of sample will be aspirated from each well.

- The sample volume you specify should be based on the cell concentration of the sample and the number of events you want to acquire.

  For example, if your sample concentration is 1,000,000 cells/mL (1,000 cells/µL) and you want to acquire 10,000 events, your sample volume should be at least 10 µL. Specifying a sample volume of 20 µL would give you plenty of leeway to acquire 10,000 events.

- The sample volume is affected by the plate-dependent dead volume. Information on dead volume for plates compatible for use with the BD FACSArray bioanalyzer can be found in the BD FACSArray Plate Definition Guide packaged with your user's guide.

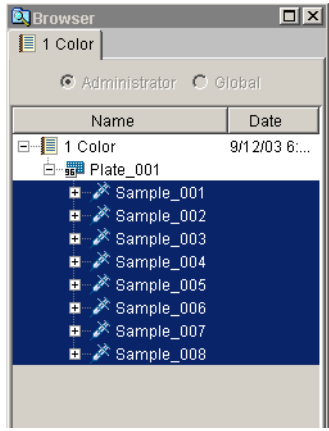
For information about other loader settings, see Inspector on page 103. For more information on sample volume, see Understanding Volumes on page 105.

Adding Keywords

This section shows you how to add keywords to your experiment.

1 In the Browser, select Samples A1–A8.

To select contiguous samples, click the first sample, hold down the Shift key, and then select the last sample.



2 Click the Edit Keywords button in the upper right section of the Plate Editor.

3 In the Editing Keywords dialog box, do the following.

- Click the Add button.

  Keyword 1 appears in the Keyword list.

- If necessary, select Numeric from the Type drop-down menu.

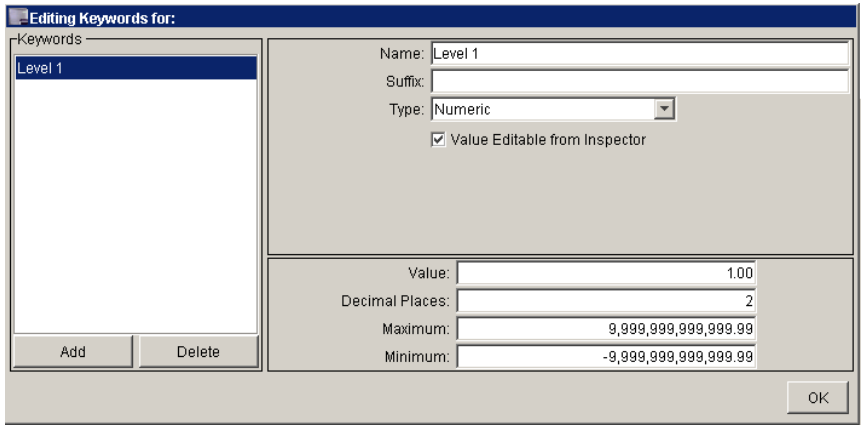
- Click to select the Value Editable from Inspector checkbox.

- Click in the Name field and enter *Level 1;* press the Enter key on the computer keyboard to save the entry.

  Level 1 appears in the Keywords list.

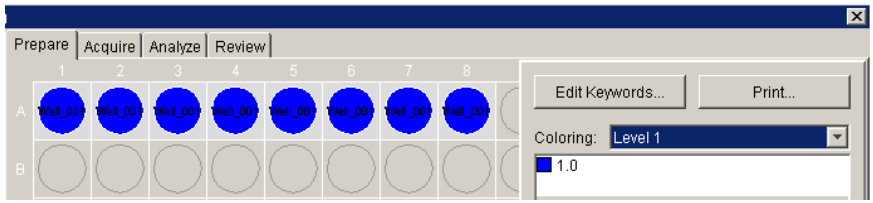
- Enter *1.00* in the Value field; press the Enter key on the computer keyboard to save the entry.

- Click OK.



4 In the Plate Editor, click the drop-down menu in the Coloring field and select the keyword *Level 1*.

In the plate view, wells 001 through 008 turn the same color, indicating they all have the same keyword.

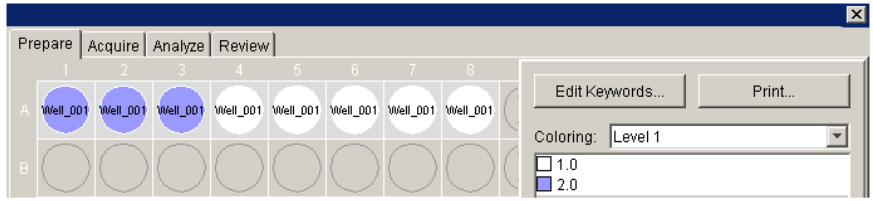


5 In the plate view, select wells A1–A3.

6 In the Well Inspector, change the value of Level 1 to 2.00; click Enter on your computer keyboard.

7 Select *Level 1* from the Coloring drop-down menu.

Wells A1–A3 turn lavender while wells A4–A8 turn white.



8 Click the drop-down menu in the Coloring field and choose Sample.

The labeled, color-coded wells appear in the plate view.

9 Click the Print button in the upper right corner of the workspace.

A print dialog appears.

10 Choose the appropriate printer and change any print options as necessary; click OK.

Choosing Acquisition Settings

1 Click the Acquire tab in the Plate Editor.

2 In the plate view, click well A1.

3 In the Acquisition Control group select the following options.

- Click the Events to Display drop-down menu and select 1,000 evts.
- If not already selected, click the Export Statistics checkbox.

4 Repeat step 1 through step 3 for the remaining wells.

Preparing for Acquisition

WARNING All biological specimens and materials coming into contact with them can transmit potentially fatal disease. To prevent exposure to biohazardous agents, handle all biological specimens and materials as if capable of transmitting infection. Dispose of waste using proper precautions and in accordance with local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

NOTICE To avoid damaging the BD FACSArray bioanalyzer, do not run samples containing particles greater than 50 µm.

Preparing Samples

You can either prepare your samples in tubes according to the appropriate reagent package insert, or develop and validate your own protocol for preparing samples directly in the 96-well plate.

Preparing the Plate

Use the printout of the plate view to transfer samples to the 96-well plate.

The first few times you run an experiment, BD recommends adding the maximum volume allowable to the first well because you will use sample from the first well to optimize instrument settings. The maximum volume varies depending on plate type. Information on dead volume for plates compatible for use with the BD FACSArray bioanalyzer can be found in the BD FACSArray Plate Definition Guide packaged with your user's guide. Make sure you have extra sample in case you need to rerun the setup wells.

Default settings for cellular templates should put cellular populations from human blood prepared using a lyse/wash method on scale. However, cell lines have different scatter characteristics, so for this application, you will need to optimize the forward scatter (FSC), side scatter (SSC), and yellow parameter settings.

Loading the Plate

⚠ **CAUTION** Movement of mechanical parts within the instrument can pinch or injure your hands or fingers. To prevent injury by moving parts, follow these precautions.

- Keep your hands away from the plate holder once you position the plate on it. The plate holder will automatically retract when you click Load.
- Keep your hands away from the plate door during instrument operation.

⚠ **NOTICE** To prevent damage to the bioanalyzer, remove any lid, film, or cover from the plate before loading onto the plate holder. Some combinations of plate and lid might not retract properly into the instrument, potentially causing damage to the plate and the plate sampler. Leaving a lid, film, or cover on the plate might cause clogs in the probe and sample line, affecting performance and possibly damaging the bioanalyzer.

1. Click the Setup tool in the application toolbar to open the Setup workspace.
2. Place the plate containing the stained samples on the plate holder.

   Position the plate so that well A1 is over the A1 mark on the plate holder.

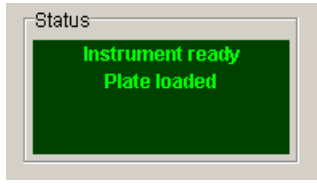
3 Click the Load button in the Plate Control group.

The plate will be retracted three-quarters of the way into the bioanalyzer.

- If a lid is detected on the plate, the plate will be ejected from the bioanalyzer, and the following message will apper on the computer monitor.

- If no lid is detected on the plate, the plate is completely retracted into the bioanalyzer.

  The Status box should display the following message.



Preparing for Optimization

Before viewing live events, keep in mind:

- In Setup mode, data is not saved. Events are displayed continuously until you stop acquisition or the volume aspirated is exhausted.
- When you click Acquire, the sample volume you specified will be aspirated and processed, and data will be saved.

- Once acquisition is in process, clicking Setup or Acquire to stop viewing live events will not return unused sample to the well.

- The bioanalyzer will aspirate the specified sample volume plus an additional 20 µL from each well.

Optimizing Instrument Settings

1. In the Select Control group, click None to deselect the wells, and then click well A1.

2. Click the Setup button in the Acquisition Control group.

   Events appear in the plots, but data is not being saved.

3. Optimize instrument settings, if necessary.

   - Adjust the FSC and SSC voltages to place the population of interest on scale in the FSC vs SSC plot.

     Adjust the signal for events displayed in plots by changing parameter voltages (electronic gain for FSC). Higher voltages increase detector sensitivity, resulting in increased signal. Lower voltages decrease detector sensitivity, resulting in decreased signal.

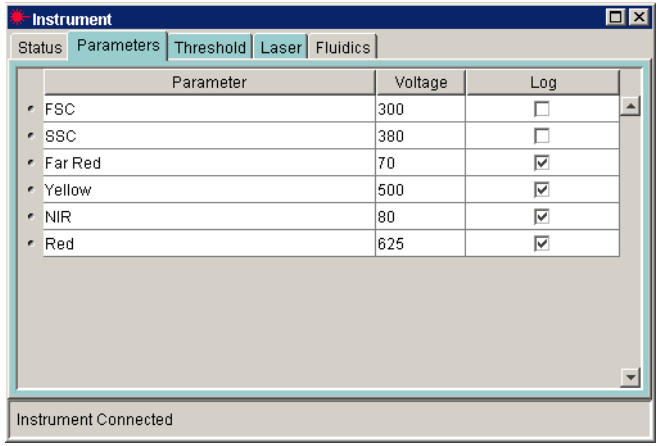
     - Click the Parameters tab in the Instrument frame to open the Parameters pane (Figure 7-18 on page 252).

     - Click in the Voltage field to edit the setting. Voltages can be adjusted from 1–1,000.

     - Press Enter on your computer keyboard to save changes.

     For more detailed information about parameters, see Parameters Tab on page 67.

Figure 7-18 Parameters tab of Instrument frame



- Adjust the threshold to eliminate noise. A threshold is a boundary below which data will not be acquired.

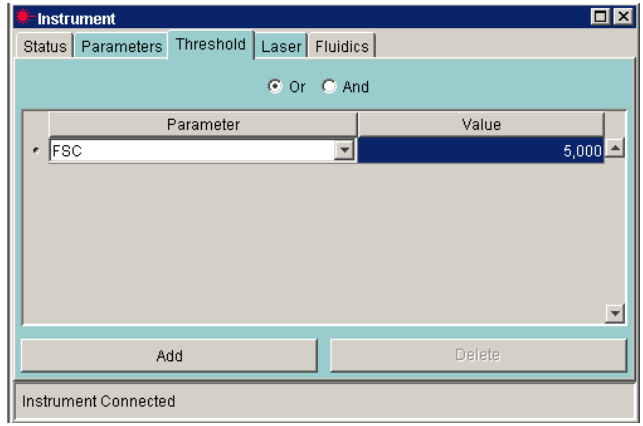
  - Click the Threshold tab in the Instrument frame to open the Threshold pane (Figure 7-19 on page 253).

  - Click in the Value field to edit the setting.

  - Press Enter on your computer keyboard to save the change.

  You can choose one or two threshold values. For more information on threshold, see Threshold Tab on page 68.

Figure 7-19 Threshold tab of Instrument frame



- Adjust the Yellow parameter voltage to ensure that both the brightest stained cells and the dim fluorescent cells remain on scale. Press Enter on your computer keyboard to save the change.

4 Click Setup to stop acquisition.

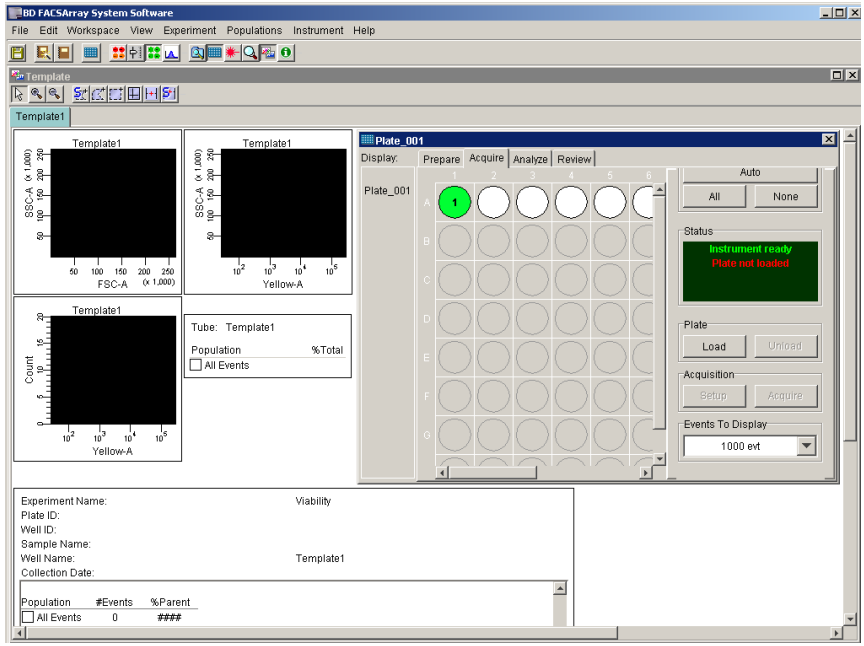
Acquiring Samples

1 Click the Acquire tool ( ) in the application toolbar to open the Acquire workspace.

2 Adjust the size of the Plate Editor so that it does not overlap plots.

The Acquire workspace should look similar to the one in Figure 7-20 on page 254.

Figure 7-20 Acquire workspace



3 In the Select Control group, click the Auto button.

All samples are selected for acquisition.

NOTICE Verify that you have enough sample in well A1 to run the sample.

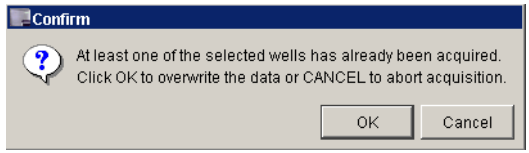
4 Click the Acquire button in the Acquisition Control group.

After a short pause, the following occurs.

- An orange ring appears in the first well selected for acquisition indicating that sample is being acquired.

- Events appear in the plots.
- Data is saved.

If any of the wells selected for acquisition already contain data, the following alert dialog appears.


Confirm
At least one of the selected wells has already been acquired.
Click OK to overwrite the data or CANCEL to abort acquisition.
OK
Cancel

- Clicking OK will begin acquisition and data will be overwritten.
- Clicking Cancel will abort acquisition.

For instructions on how to reacquire a well without overwriting existing data, see Reacquiring a Well on page 238.

What to Monitor During Acquisition

1 Monitor the Status box for error messages.

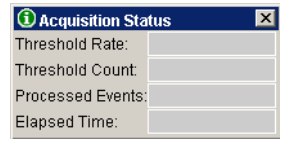
If an instrument error occurs during acquisition, a message will be displayed on the Status box in the Acquire workspace.

2 To view detailed error information, open the Instrument frame by clicking the Instrument tool ( ) in the application toolbar and click the Status tab.

For more information on the Status tab of the Instrument frame, see Status Tab on page 66. See page 337 for troubleshooting information.

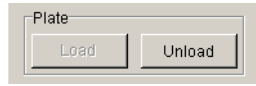
3 Click the Acquisition Status tool ( ) in the application workspace to open the Acquisition Status frame.

The Acquisition Status frame is a useful tool to monitor whether events are being detected and processed during acquisition.



Unloading the Plate

1 Once the plate has been acquired, click the Unload button in the Plate Control group to eject the plate from the plate sampler.



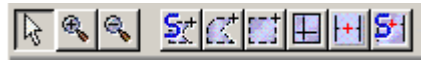
2 Remove the plate from the plate holder.

Data Analysis

Use the following instructions to analyze your data.

1 Click the Analyze tool in the application toolbar to open the Analysis workspace.

2 Use the template toolbar to set any gates for analysis.

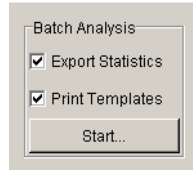


3 Review data for a few wells to ensure gates created encompass the appropriate population.

Because the same gates are applied to all wells in the experiment, this step ensures that gates created are appropriately set to analyze the data in most wells.

4 Choose to export statistics and print the templates by clicking the two radio buttons in the Batch Analysis Control group.

5 Click start.



The data files will be analyzed using the predefined analysis gates.

Data Review

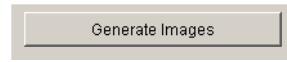
You can use the review feature to view data in a selected plot to do the following plate-based assessment of data.

- View images of data
- Quickly verify a Snap-To gate is enclosing the population of interest
- Screen for specific populations

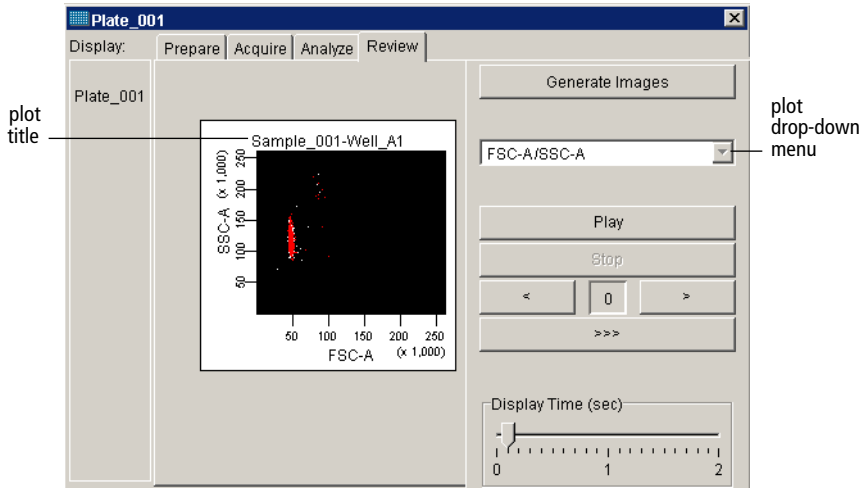
Use the following instructions to review the data analysis.

1 Click the Review tab in the Plate Editor.

2 Click the Generate images button in the Review Control group.



The software will quickly convert the results in the Template view to a series of images of each analyzed plot. Once the conversion is complete, the remaining buttons in the Review Control group become enabled.



NOTICE The plot title displays the sample and well name to identify the data.

3 Choose the plot to display from the drop-down menu.

4 Use the forward (>) and reverse (<) buttons to display plot images from different wells.

Alternatively, choose the Display Time, and then click Play to view plot images in succession.

Advanced Gating Features

BD FACSArray system software provides gating tools to support multicolor data analysis. The following tutorial demonstrates some of the software's advanced gating features.

In this example, lysed whole blood cells were stained with CD19 PE-Cy7, CD16+CD56 PE, CD45 APC-Cy7, and CD3 APC. If you want to practice gating by following the steps in this section, import the *Gating Tutorial* experiment from the BD Export folder (D:\BDExport\Gating Tutorial\AdvGatingTutorial).

Importing the Tutorial Experiment

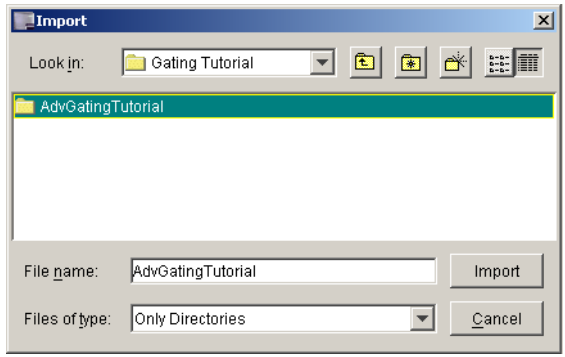
1 If not already open, launch BD FACSArray system software.

2 Choose File > Import Experiments.

3 Navigate to D:\BDExport\Gating Tutorial; double-click the AdvGatingTutorial folder.

The experiment is added to the database and appears in the Browser. The experiment contains setup and sample data.

4 Select the AdvGatingTutorial folder; click Import.



Displaying Data

1 In the Browser, double-click the AdvGatingTutorial experiment to open it.

2 Click the Analyze tool ( ) in the application toolbar to display the Analyze workspace.

3 Verify the Analyze tab in the Plate Editor is displayed.

4 Click in well F1 to display data for the well in the Template view.

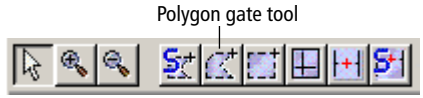
If the plate view is not displayed, click the plate icon ( ) in the application toolbar to display it.

Gating on Monocytes and Neutrophils

1 If necessary, change one of the plots to display CD45 vs SSC.

- Click the parameter name on the plot.
- Select the appropriate parameter from the popup menu.

2 Click the Polygon gate tool in the Template toolbar.



3 In the CD45 vs SSC plot, create a gate around the monocytes and neutrophils.

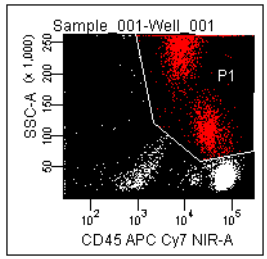
- Click inside the plot to position the first vertex of the polygon region.
- Move the cursor to the next position and click to add another vertex.

  A solid line appears connecting the two vertices.

  To restart or cancel the region after setting the first vertex, click outside the plot view.
- Continue moving and clicking around the population until you reach the first vertex.
- Click on the first vertex to complete the polygon.

  You can also double-click the mouse button to set the last vertex and complete the polygon.

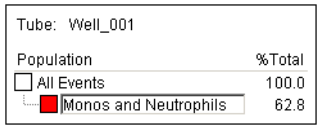
  When you have completed the region, it appears as a solid outline with handles at each corner of the polygon. These handles indicate the region is selected. Click and drag any of the handles to resize the region. Click on the border of the region to drag it to a new position.
- Move the region label so that it does not obscure any data.



☑ **Tip** Draw the gate as close as possible to the plot's borders without touching them. Once created, drag the gate to the plot's borders to ensure events at the edges are not missed.

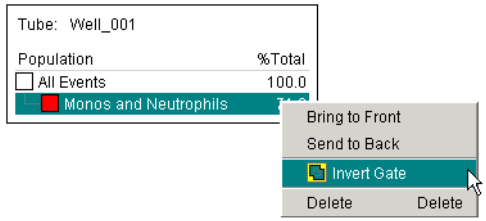
4 Click the P1 label in the Population Hierarchy and rename it *Monos and Neutrophils*.

The Population Hierarchy is located to the right of the second set of plots.

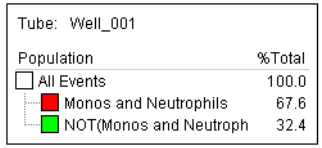


Inverting the Gate

1 Right-click the *Monos and Neutrophils* label in the Population Hierarchy and choose Invert Gate.

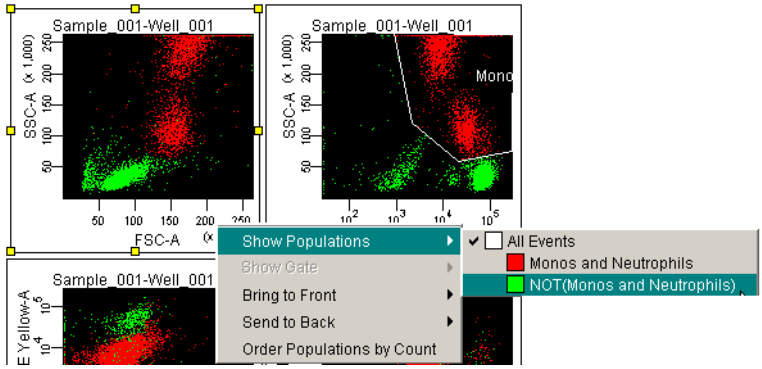


A new population appears in the hierarchy. The population contains all events NOT in the *Monos and Neutrophils* gate.

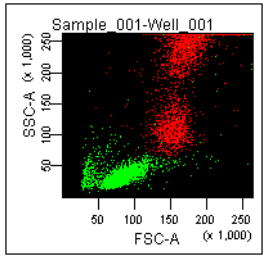


2 If necessary, change one of the plots to display FSC vs SSC.

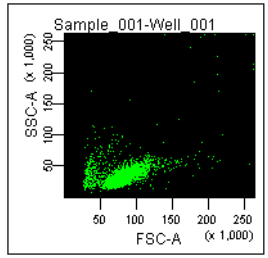
3 Right-click the FSC vs SSC plot border and choose Show Populations > Not (Monos and Neutrophils).



The monocytes and neutrophils disappear from the FSC vs SSC plot, making it easier to draw a gate around the lymphocytes.

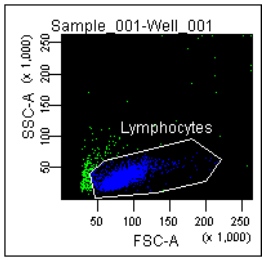


Ungated

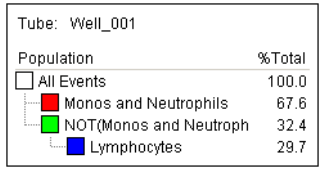


Gated

4 Draw a polygon gate around the lymphocytes; change the name of the population to *Lymphocytes* in the Population Hierarchy.



Since the lymphocyte population was defined in a plot showing only NOT Monos and Neutrophils, the new population becomes a subset of the NOT Monos and Neutrophils population and is further indented in the Population Hierarchy.



Gating on CD3+ T cells

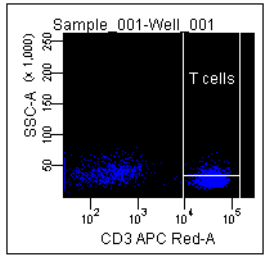
1. If necessary, change one of the plots to display CD3 vs SSC.
2. Right-click the CD3 vs SSC plot border and choose Show Populations > Lymphocytes.
3. Draw an interval gate around the CD3+ T cells and name the new population *T cells*.
   - Click the Autointerval gate tool in the Template toolbar.
   - Click inside the plot to position the first vertex of the interval gate.

     To restart or cancel the region after setting the first vertex, click outside the plot view.
   - Move the cursor to expand the gate to include the CD3+ T cells.

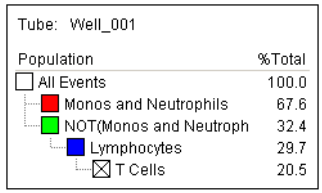
     The gate appears as a solid line connecting the two vertices with handles at each end.

     These handles indicate the region is selected. Click and drag any of the handles to resize the region. Click on the border of the region to drag it to a new position.

- Move the region label so that it does not obscure any data.
- Click the label in the Population Hierarchy and rename it *T cells*.



Since the T cells population was defined in a plot showing only lymphocytes, the new population becomes a subset of the lymphocytes and is further indented in the Population Hierarchy.

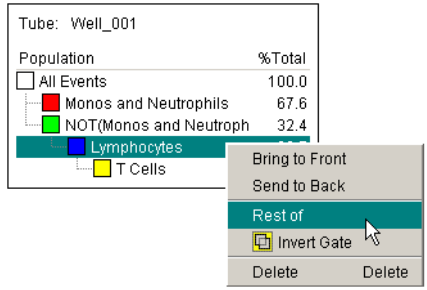


NOTICE Interval gates, normally used on histograms, work equally well on dot plots (in a vertical direction). Note that events within an interval gate retain the color of their parent population.

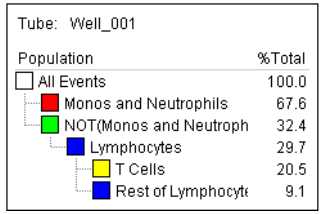
4 Choose a color for the T cell population.

- Double-click the T cells color box in the Population Hierarchy.
- Select a new color from the color palette that appears.

5 Right-click the *Lymphocytes* label in the Population Hierarchy and choose *Rest of* from the menu that appears.



Rest of Lymphocytes appears in the Population Hierarchy. Any events not classified as T cells is counted in the Rest of Lymphocytes population.



6 Select another color for the Rest of Lymphocytes population in the Population Hierarchy.

Creating a Quadrant Gate

1 If necessary, display CD19 vs CD16+CD56 in one of the plots.

2 Right-click the CD19 vs CD16+CD56 plot border and choose Show Populations > Rest of Lymphocytes.

3 Click *Rest of Lymphocytes* in the Population Hierarchy.

4 Click the Quadrant Gate tool.

5 Click in the CD19 vs CD16+CD56 plot and position the quadrants so they separate the populations.

6 Click the label in the Population Hierarchy and rename Q1 *NK cells*.

NK cells are CD3 negative, CD16+CD56 positive.

7 Choose a color for the NK cells population.

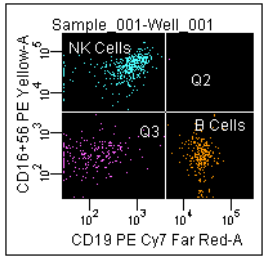
- Double-click the NK cells color box in the Population Hierarchy.
- Select a new color from the color palette that appears.

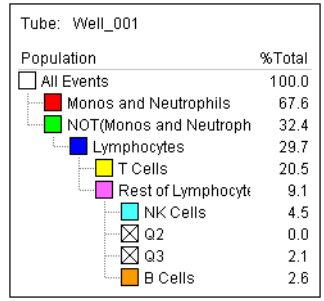
8 Click the label in the Population Hierarchy and rename Q4 *B cells*.

9 Choose a color for the B cells population.

- Double-click the B cells color box in the Population Hierarchy.
- Select a new color from the color palette that appears.

Your plot and population hierarchy should look similar to the following figure.





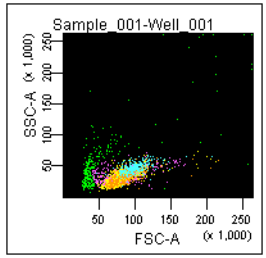
You have completed analysis of T, B, and NK cells.

Choosing Populations to Display

BD FACSArray system software provides several options for you to display data. Perform the following tutorial to practice using the display options.

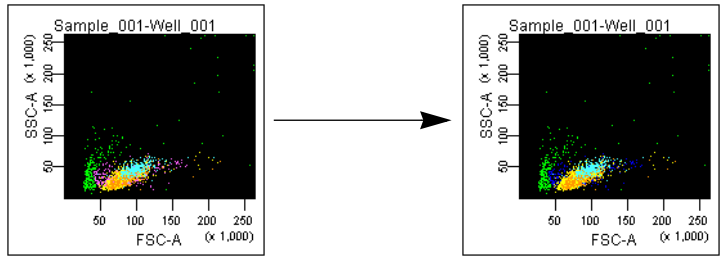
1 Right-click the FSC vs SSC plot border and choose Show Gate > Lymphocytes.

The lymphocyte gate border becomes hidden and is no longer displayed on the FSC vs SSC plot.



2 Right-click the FSC vs SSC plot border and choose Send to Back > Rest of Lymphocytes.

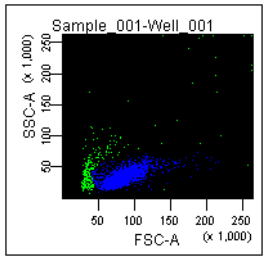
The Rest of Lymphocytes population is no longer visible in the plot. Notice that the purple events in the *before* plot are no longer visible in the *after* plot.



before after

3 Right-click the FSC vs SSC plot border and choose Bring to Front > Lymphocytes.

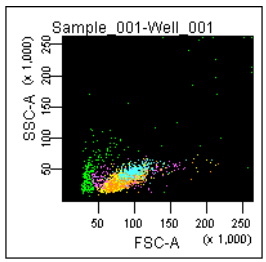
The lymphocytes population color is displayed on top of all other population colors.



4 Right-click the FSC vs SSC plot border and choose Order Populations by Count.

This feature allows you to display populations based on the number of events. This ensures that rare events are drawn on top of other events.

The rare event population appears in front of the others.



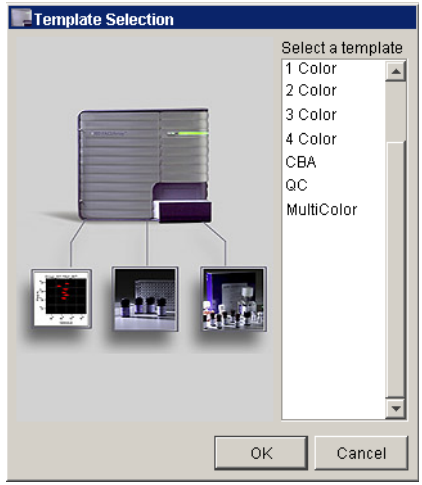
Running Multiple Assays on One Plate

You can run different assays on one plate, if needed. The following tutorial shows you how to create and run a four-color and a two-color assay that were prepared on a single plate.

Alternatively, you could set up the first experiment using the Experiment Wizard and the second experiment manually.

Creating the First Experiment

1 Click the New Experiment tool ( ) to open the Template selection view.



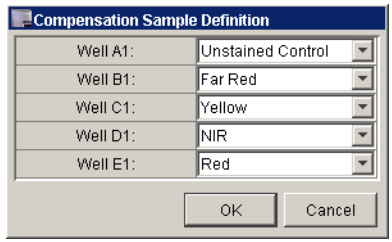
2 Select a template.

For this example, choose the 4-color template.

3 In the plate view, click well A1, hold down the shift key, and click well E1.

4 In the Layout for Plates control group, click Add Setup.

The following dialog appears.



5 Verify the appropriate parameters are specified for each well.

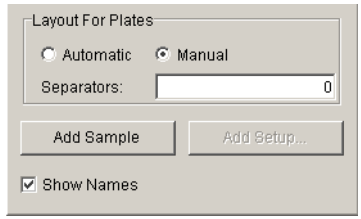
6 Click OK.

7 Divide wells into samples.

- Select well A2, hold down the shift key, and click well H2.

  All wells in the column become selected.

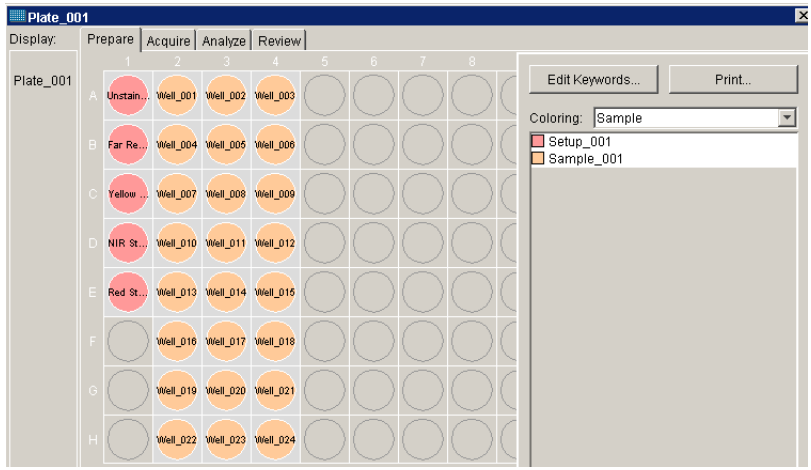
- Click the Add Sample button in the Layout for Plates Control group.



- Select well A3, hold down the shift key, and click well H4; click Add Sample.

- Select well A4, hold down the shift key, and click well H3; click Add Sample.

Alternatively, you could select wells A2 through H4 (columns 2 through 4), and then click Add Sample.



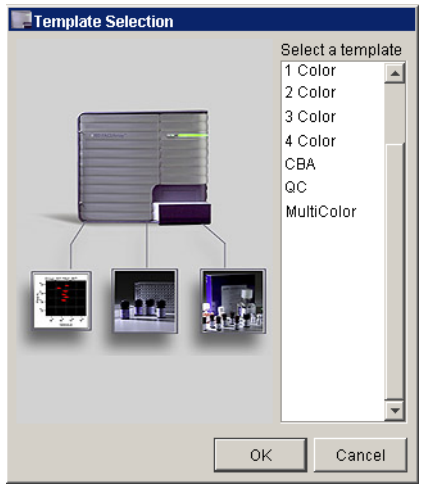
8 If needed, adjust loader and acquisition settings per well.

The sample wells retain the default loader and acquisition settings from the 4-color template.

Creating the Second Experiment

1 Click the New Experiment tool ( ) to open the Template selection view (Figure 7-21 on page 272).

Figure 7-21 Template Selection view



2 Select a template.

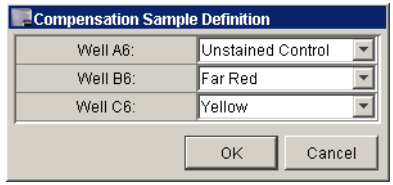
For this example, choose the 2-color template.

3 In the plate view, click well A6, hold down the shift key, and click well C6.

The wells become selected.

4 In the Layout for Plates control group, click Add Setup.

The following dialog appears.

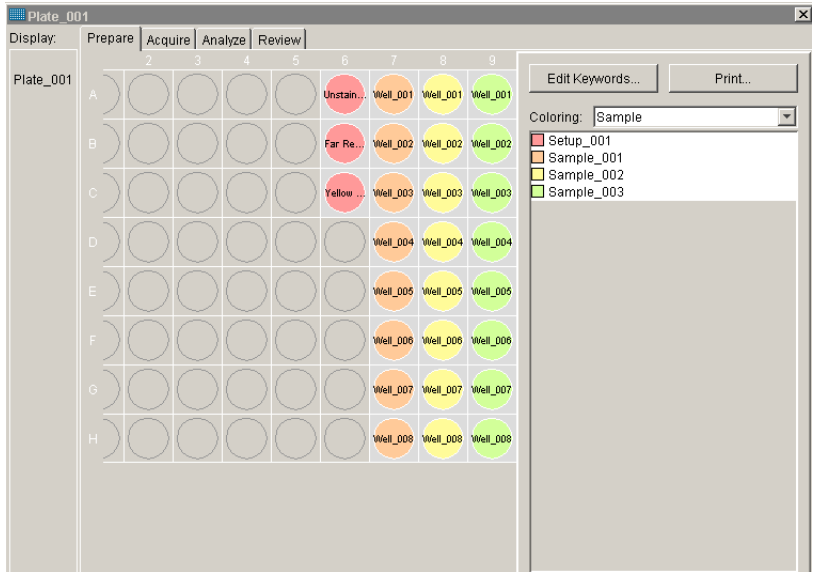


5 Verify the appropriate parameters are specified for each well.

For this example, choose Unstained Control, Yellow, and Red.

6 Click OK.

7 Repeat the process for dividing wells into samples as shown in step 7 on page 270 using wells A7–H7, and A8–H8.



8 In the Browser, close the experiment.

Acquiring and Analyzing Data

1 In the Browser, open the first experiment.

2 Load the plate onto the plate sampler, and then click Load in the Plate Control frame.

3 Optimize instrument settings, if needed.

See Optimizing Instrument Settings on page 227.

4 Save and analyze data.

   See Acquiring Samples on page 233 and Data Analysis on page 235.

5 Close the first experiment.

6 Open the second experiment.

7 Repeat step 3 on page 273 to optimize instrument settings.

8 Repeat step 4 to acquire and analyze data.

8

Data Management

The following topics are covered in this chapter:

- Working with BD FACSArray Data on page 276
- Exporting Data on page 281
- Exporting and Importing Experiments on page 286
- Using the BD FACSArray Data Manager on page 289

Working with BD FACSArray Data

BD FACSArray system software does not generate separate files for experiments, instrument settings, and data. The software stores and accesses all experimental data through the database.

Any changes to an open Experiment, related Browser elements, or template are automatically saved when you close an Experiment, quit the application, or click the Save tool ( ) in the Workspace toolbar. The database contains a record of all Browser items, template elements, Experiment settings, and Instrument Control settings.

Event data is saved separately in FCS 3.0 floating-point format.* A green icon ( ) to the left of the well name in the Browser signifies that data has been saved for that well. To analyze data in another software application, data must be exported using the FCS Export option in the software (see Exporting FCS Files on page 282). To analyze data with BD FACSArray software on another workstation, data can be exported using the Export Experiment option (see Exporting Experiments on page 287).

⚠ **NOTICE** To ensure that data can be accessed by the software, do not move, rename, or delete the BDFACS.db file, BDFACS.log file, or BDData folder inside the BDDatabase folder. Do not change the name of any file or folder within the BDData folder.

⚠ **NOTICE** FCS files within an exported experiment do not contain the same information as FCS files exported using the Export FCS command. Do not import FCS files within an exported experiment using the Import FCS files command. To prevent confusion, BD Biosciences recommends storing exported experiments in a folder separate from exported FCS files.

* For more information, see http://www.isac-net.org.

Maintaining Data

Because all data is saved in a database, the database can fill up the hard drive. It is important to maintain the database by keeping the size below recommended limits, backing up the database on a regular basis, and deleting Experiments that are no longer needed.

⚠ **NOTICE** To preserve the integrity of your data, follow these precautions:

- Monitor the disk capacity (see Verifying Database Size on page 279). The amount of hard disk space required for the database must not exceed 15 GB (additional space reserved for a backup copy). When you are approaching the limit, delete unneeded Experiments or back up Experiments and store them in another location.

- Back up the database on a regular basis.

- Be sure you have successfully completed an acquire, print, save, or delete operation before closing the BD FACSArray application. Because some operations take time to complete, you risk losing unsaved data if you terminate the application prematurely.

- Defragment the hard disk on a regular basis (ie, weekly). Diskeeper® defragmentation software is installed on workstations purchased through BD Biosciences. To run the program, choose Start > Programs > Executive Software Diskeeper. You can program the software to defragment the disk automatically on a preset schedule.

  ⚠ **NOTICE** Data loss can occur if the defragmentation process is abruptly interrupted. BD Biosciences recommends that you back up your data before running the defragmentation procedure and allow sufficient time to defragment the drive.

- BD Biosciences recommends that you disable sleep mode on your computer monitor when running BD FACSArray system software. Pressing keys while the system is waking up could execute an unwanted command that might result in loss of data without your knowledge.

Table 8-1 summarizes options for maintaining the BD FACSArray database using the tools provided with the software. Follow the procedures established in your laboratory for scheduling data backups. General guidelines are provided in the table.

Table 8-1 Data management options

Option	Function	When to Do	See...
Export FCS data	Exports data as FCS 2.0 or 3.0	When analyzing data in other compatible software applications	page 282
Export CBA data	Exports data from standards and samples for analysis in BD CBA software	Analyzing data in BD CBA software	page 284
Export statistics	Exports statistics from a single well	As needed for further data analysis with other software	page 137
Export Template elements	Exports elements (ie plots and Population Hierarchy views as JPEG files	As needed for creating publications and other uses with graphics or word processing software	page 139
Export Experiments	Exports an experiment and related elements to a specified location with the option to remove them from the current database on completion of export	• When sharing data with users who have BD FACSArray system software • When necessary to free up disk space • As a means for backing up data	page 287
Delete Experiments	Frees up space in the database and hard drive by removing unnecessary or outdated components	When necessary to free up disk space	page 281

Table 8-1 Data management options (continued)

Option	Function	When to Do	See...
Import Experiments	Imports an experiment from a specified location	• When reanalyzing an experiment that was previously exported and deleted from the database • When sharing data with users who have BD FACSArray system software	page 288
Back up database	Copies the current database to the specified drive	Before installing a software upgrade	page 291

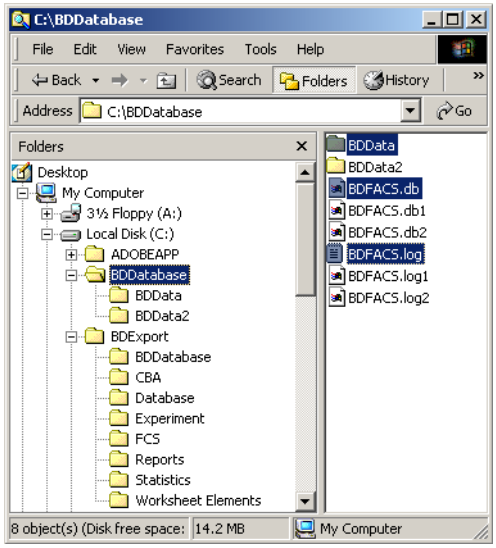
Verifying Database Size

The database should not exceed 15 GB. (The remaining space is reserved for a backup copy of the database.) To determine the size of the database, do the following.

1. Use Windows Explorer to view the contents of the BDDatabase folder (Figure 8-1 on page 280).

2. Select the BDData folder, the BDFACS.db file, and the BDFACS.log file.

Figure 8-1 Contents of Database folder



3 Right-click the selected items and choose Properties.

A window appears showing the size of the selected components.

⚠ **CAUTION** If the size is approaching 15 GB, delete or archive (export and delete) experiments to free up space on the hard drive.

Deleting Experiments

Do the following to delete unneeded experiments.

⚠ **NOTICE** Deleting experiments also deletes any associated event data. Export FCS files before deleting experiments.

1. Export the experiments you want to save.

   See Exporting Experiments on page 287 for instructions.

2. In the BD FACSArray Browser, select the experiments you want to remove.

   - To select multiple contiguous elements, click on the first element; then hold down the Shift key while clicking on the last element to be removed.
   - To select multiple discontiguous elements, hold down the Control key while clicking on each element to be removed.

3. Press the Delete key.

   Alternatively, right-click selected elements and choose Delete from the contextual menu.

Exporting Data

Data can be exported to an FCS file that can be read by other BD software and third-party applications. Files can be exported in FCS 2.0 or 3.0 format.

Instrument settings are written to the FCS file but cannot be opened as a separate file in BD CellQuest Pro software. Instrument settings are not transferable between different cytometers.

Use the Export command in the File menu to export experiments, FCS files, CBA files, statistics, and elements from the Template view.

Exporting FCS Files

Follow the steps in this section to export non–BD CBA FCS files for use with other applications. See Exporting BD CBA Data on page 284 for specific instructions on exporting BD CBA data.

NOTICE Original data remains in the database after exporting FCS files.

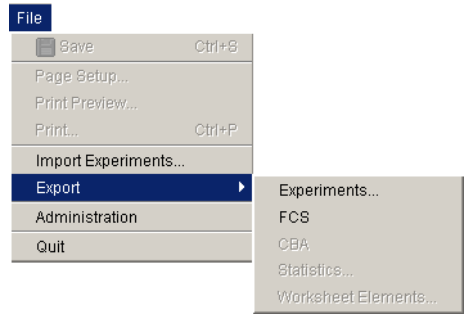
1. In the Browser, select the experiment, plate, sample, or well(s) to be exported.

   Data can be exported from open experiments; multiple items can be selected for exporting at one time. Individual FCS files will be generated for each well in a selected sample or experiment.

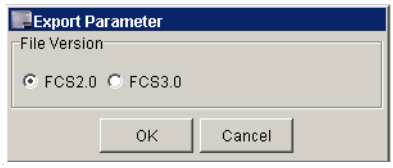
2. Choose File > Export > FCS.

   Alternatively, right-click the selected item(s) and choose Export FCS.

   Only well keywords are exported with FCS files; sample, plate, and experiment keywords are not exported.



3. Specify the FCS file version.



   When multiple files are selected, options apply to all exported files.

- Select FCS 2.0 file format

  To make an exported file compatible with BD CellQuest Pro software, export as FCS 2.0 file. The file will be saved in 1024 resolution in either log or linear depending on how it was acquired. The file will contain compensated data.

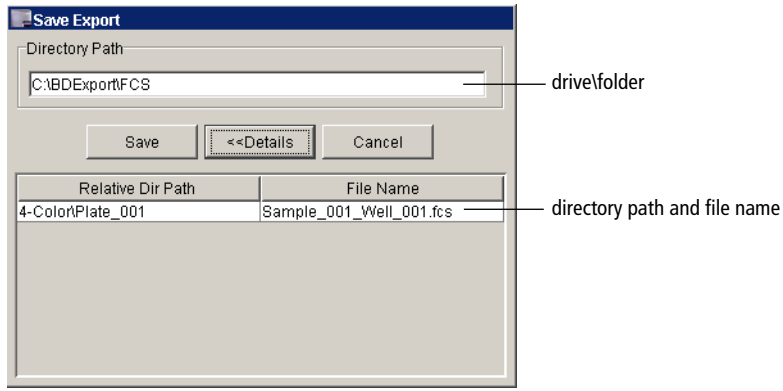
☑ **Tip** To check whether the data was saved in log or linear mode, go to the Setup workspace, select the well in the plate view, and open the parameters tab of the Instrument frame.

- Select FCS 3.0 file format.

  The file will be saved in 262,143 resolution in either log or linear depending on how it was acquired. The file will contain uncompensated data.

4 Click OK.

5 In the Save Export dialog box, verify the file storage location.



BD FACSArray software does not automatically create a folder for exporting FCS files, and you cannot create a new folder using the Save Export dialog. You must create the folder prior to exporting the data.

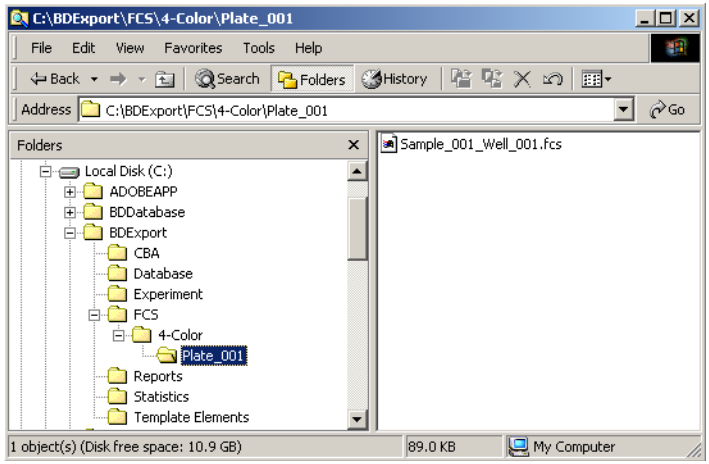
- Enter a different logical drive name or folder name if you want to change the file storage location.

- Click the Details button to view the directory path and file name for each exported well. Note that the file names are taken from the well names in the Browser and cannot be changed.

☑ **Tip** To avoid confusion, store exported FCS files in a folder different from exported experiments. For this example, a folder was created with the same name as the experiment

6 Click Save to export the files.

7 Verify the location of your exported files.



Exporting BD CBA Data

When you use the BD FACSArray Experiment Wizard to create a CBA experiment, the software inserts a specimen type keyword (either Sample or Standard). The software then uses this keyword to determine where to put the FCS files, either in a Samples folder or a Standards folder.

If you create a CBA experiment manually (even if you use the CBA template), the files cannot be automatically place in the appropriate folder. Therefore, for ease of use, use the Experiment Wizard to create your CBA experiments.

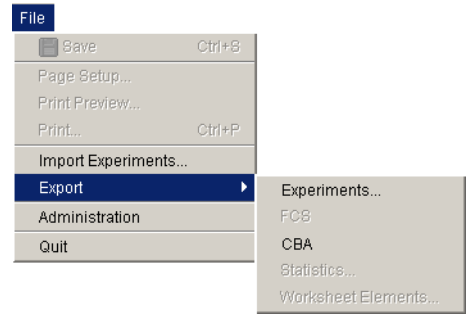
Use this procedure to export BD CBA data into FCS 2.0 format.

1 Open the CBA experiment you want to export.

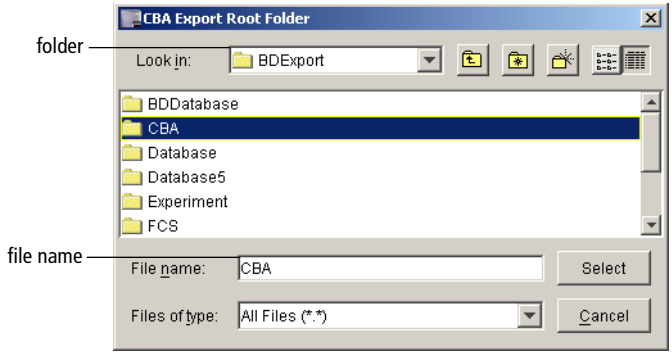
In the Browser, double-click the experiment to open it.

2 Choose File > Export > CBA.

Alternatively, right-click the experiment elements and choose Export CBA.



3 In the CBA Export Root Folder dialog box, verify the file storage location.



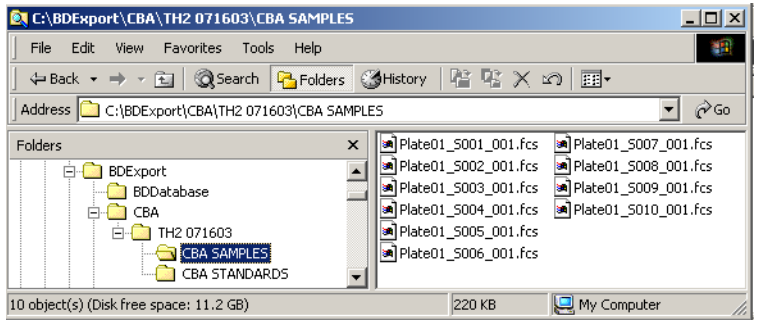
Enter a different folder name if you want to change the file storage location.

☑ **Tip** To avoid confusion, store exported CBA data in a folder different from exported experiments and exported FCS files.

4 Click Select to export the files.

BD FACSArray system software will export the BD CBA data into either a CBA Standards or a CBA Samples folder within the selected folder.

5 Verify the location of your exported files.



Exporting and Importing Experiments

Experiments can be exported to the hard drive or another storage location using the Export option on the File menu. When the export is complete, you can choose to have the software remove the exported experiment from the browser, which removes it from the database, frees disk space, and can improve system performance.

An exported experiment contains all Browser elements and their hierarchical structure. Experimental elements are exported in an XML file, while experimental data is exported in either FCS 2.0 or FCS 3.0 file format. To read the contents of the experiment, import it back into BD FACSArray software using the Import Experiments command.

⚠ **NOTICE** FCS files within an exported experiment do not contain the same information as FCS files exported using the Export FCS command. Do not import FCS files within an exported experiment to third-party applications. To prevent confusion, BD Biosciences recommends storing exported experiments in a folder separate from exported FCS files.

⚠ **NOTICE** BD FACSArray experiments cannot be imported into BD FACSDiva software.

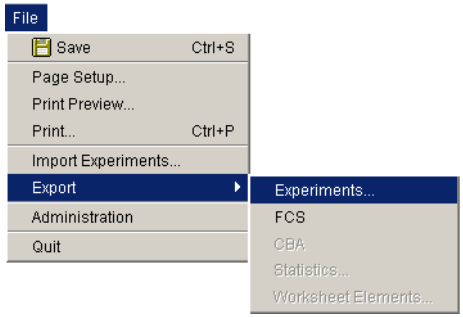
Exporting Experiments

1 If an experiment is open, close it.

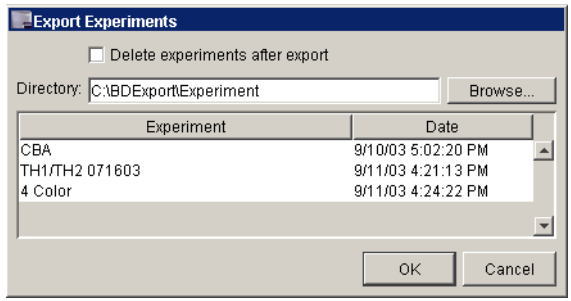
NOTICE Close the experiment by double-clicking the experiment icon.

2 Select one or more Experiments in the Browser.

3 Choose File > Export > Experiments.



4 Make appropriate selections in the Export Experiments dialog box.



BD FACSArray software does not automatically create a folder for exporting experiments, and you cannot create a new folder using the Save Export dialog. You must create the folder prior to exporting the data.

- Verify that the experiments listed are those you intended to export. If not, click Cancel and repeat steps 1 through 3.

- Select the *Delete experiments after export* checkbox if you want to delete the listed experiments from the Browser after export.

- Specify the directory where the experiments will be stored. Enter the directory path by typing, or click the Browse button and locate the storage location in the location dialog box that appears.

☑ **Tip** To avoid confusion, store exported experiments in a folder separate from exported FCS files.

5 Click OK.

The export process begins. If the *Delete experiments after export* checkbox was selected, each experiment will be deleted from the Browser immediately after its successful export.

Importing Experiments

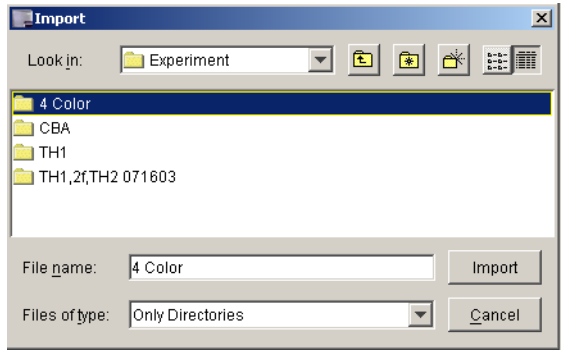
Experiments can be imported using the Import Experiments command. If an identically named experiment already exists in the Browser, the imported experiment is appended with _00*x*, where *x* is the next consecutive number for experiments of the same name.

Only BD FACSArray Experiments can be imported using this command.

1 Choose File > Import > Experiments.

2 Locate the experiment to be imported in the dialog box that appears (Figure 8-2 on page 289).

Figure 8-2 Import dialog



3 Select the folder containing the required experiment and click Import.

If you select a folder that contains only data, a warning message appears. Select only folders containing valid exported BD FACSArray experiments.

The imported experiment is added to the database and appears in the Browser.

Using the BD FACSArray Data Manager

The BD FACSArray Data Manager is installed during BD FACSArray software installation. It can be used to back up or archive BD FACSArray data, restore the database, or retrieve archived experiments.

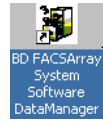
⚠ **CAUTION** Do not relocate the BD FACSArray Data Manager or run it from a batch file located outside the BD FACSArray application directory.

To launch Data Manager, do the following.

1 If not already closed, quit the BD FACSArray application.

NOTICE The BD FACSArray Data Manager cannot function when BD FACSArray software is running.

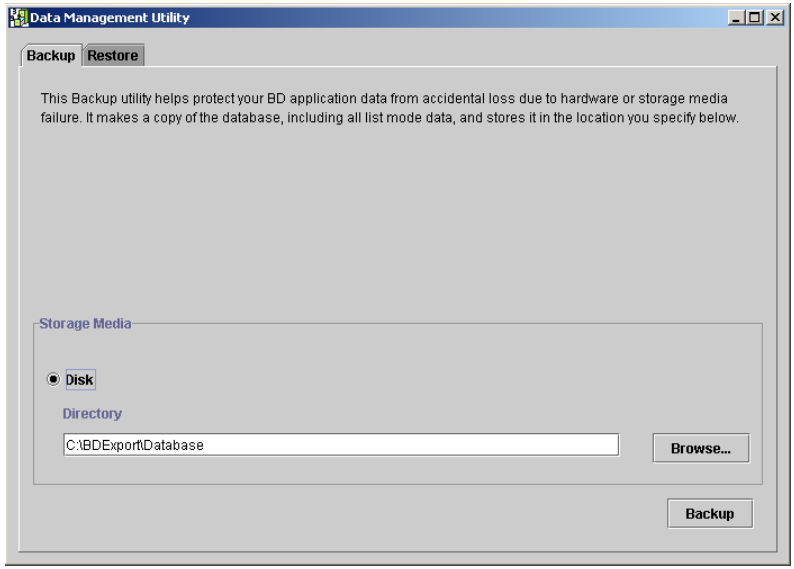
2 Launch BD FACSArray Data Manager by double-clicking the shortcut on the desktop.



shortcut icon on desktop

The following window appears.

Figure 8-3 BD FACSArray Data Management window


Data Management Utility
Backup
Restore
This Backup utility helps protect your BD application data from accidental loss due to hardware or storage media failure. It makes a copy of the database, including all list mode data, and stores it in the location you specify below.
Storage Media
Disk
Directory
C:\BDExport\Database
Browse...
Backup

The Data Manager window has two tabs. Click on the appropriate tab to initiate the following actions.

Tab	Action	See...
Backup	Create a backup copy of the BDFACS database on the specified drive.	the following section
Restore	Replace the current database with a backup copy from the specified drive.	page 294

Backing Up the Database

During a backup, the BD FACSArray Data Manager copies the current BDFACS database and associated list-mode data to the specified disk. If the workstation is connected to a network, files can be backed up directly to a mapped network drive or other storage media.

⚠ **NOTICE** Before backing up the database, make sure you have adequate storage space on the backup media (network or hard drive). Exceeding the capacity of the hard disk can result in system errors and potential data loss.

⚠ **NOTICE** It is strongly recommended that you back up files to a hard disk or network device other than the one containing the database. If you back up to the disk containing the database and the hard disk crashes, both the BD FACSArray database and the backup copy will be lost.

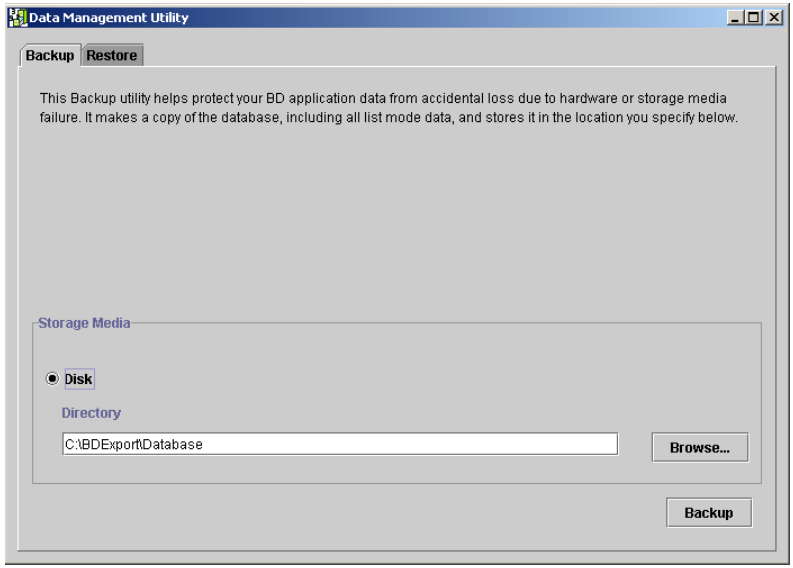
NOTICE The following option should not be used to free up space on the hard drive, because the original files are retained.

1. Launch the Data Manager application.

   See Using the BD FACSArray Data Manager on page 289. The Data Manager window appears with the Backup tab displayed.

2. If not already open, click the Backup tab of the BD FACSArray Data Manager (Figure 8-4 on page 292).

Figure 8-4 Backup tab of Data Management Utility


Data Management Utility
Backup
Restore
This Backup utility helps protect your BD application data from accidental loss due to hardware or storage media failure. It makes a copy of the database, including all list mode data, and stores it in the location you specify below.
Storage Media
Disk
Directory
C:\BDExport\Database
Browse...
Backup

3 Verify or change the path of the backup folder in the Directory field.

To change the path, click the Browse button. After mapping a network drive, files can be backed up directly to the network. See Mapping a Network Drive on page 294.

☑ **Tip** BD Biosciences recommends saving each backup in a separate folder and not replacing a previous backup. To prevent confusion, create a new, dated folder for each database backup. By entering a new folder name at the end of the directory path, the folder will be created during the backup operation.

4 Click Backup.

A progress box appears, and a message is shown when the backup is complete.

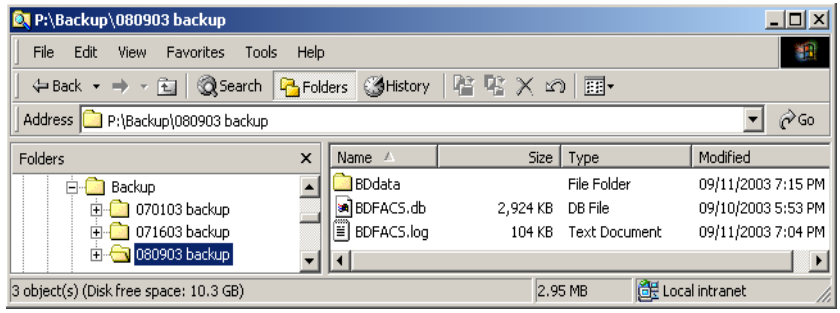
The following message appears if the folder you chose already exists.

Figure 8-5 Existing folder message

Database
This folder already exists and may contain similarly named experiments. Would you like to replace the existing files?
OK
Cancel

5 Click OK to close the dialog and select another folder.

6 Verify the presence of the backup files in the specified location.



Data Manager adds three items to the specified folder during a backup:

- a copy of the BDFACS.db database
- a copy of the BDFacs.log
- a copy of the BDData folder containing all FCS files in the database at the time of backup

Mapping a Network Drive

If your workstation is connected to a network, use the following instructions to create a network drive. Once the network is mapped, you can view the contents of the drive by clicking its icon in Windows Explorer or in My Computer. Files can be backed up directly to a mapped network drive using the BD FACSArray Data Manager.

1 Launch Windows Explorer.

   Choose Start > Programs > Accessories > Windows Explorer.

2 Choose Tools > Map Network Drive.

3 Choose a Drive letter from the Drive drop-down menu.

4 Enter the path of the network backup folder in the Folder field, ie, *servername**foldername*.

   Alternatively, click the Browse button and navigate to the required folder.

5 Click Finish.

Restoring a Database

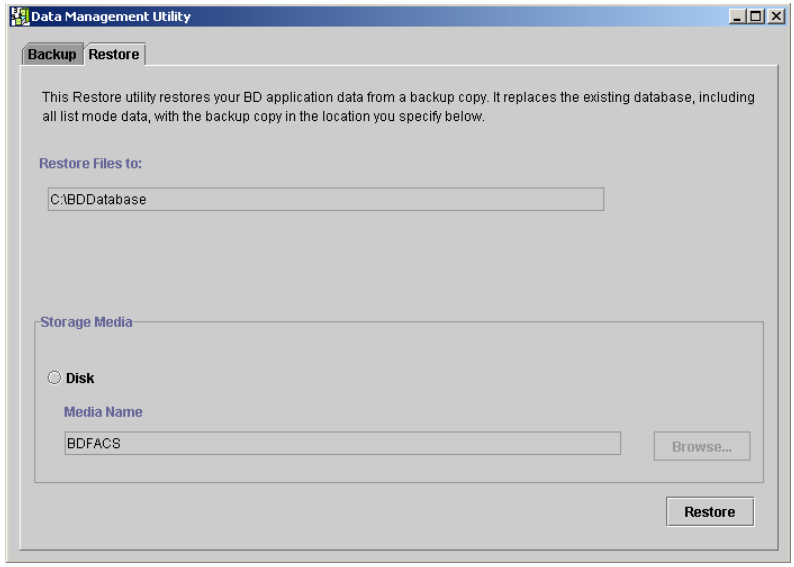
Use the Restore tab of the BD FACSArray Data Manager to replace the existing database with a backup copy from a disk.

⚠ **NOTICE** During a data restore, the current database is overwritten by the backup copy. Once the restore is in progress, it cannot be stopped or cancelled. To save your current data, make a backup before restoring a previous database.

1 Launch the Data Manager application.

   See Using the BD FACSArray Data Manager on page 289. The Data Manager window appears with the Backup tab displayed.

2 Click the Restore tab.

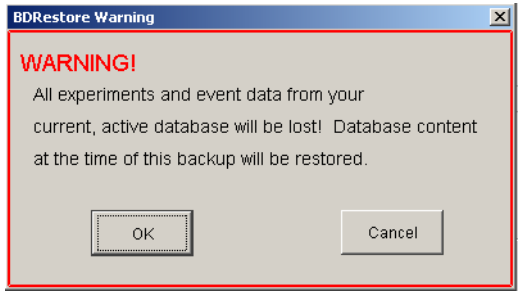

Data Management Utility
Backup
Restore
This Restore utility restores your BD application data from a backup copy. It replaces the existing database, including all list mode data, with the backup copy in the location you specify below.
Restore Files to:
C:\BDDatabase
Storage Media
Disk
Media Name
BDFACS
Browse...
Restore

3 Verify or change the path to the backup folder in the Directory field.

To change the path, click the Browse button.

4 Click Restore.

The following message appears.


BDRestore Warning
WARNING!
All experiments and event data from your current, active database will be lost! Database content at the time of this backup will be restored.
OK
Cancel

5 Click OK to continue.

The BD FACSArray Data Manager verifies that the user has logged in as the Administrator. If the verification passes, the BD FACSArray Data Manager restores the backup files to the D:\BDDatabase directory.

A progress box appears, and then a message is shown when the backup is complete.

NOTICE If an error occurs during this phase of the Data Manager Restore process, Sybase Adaptive Server Anywhere might have to be restarted manually. Call BD Biosciences for instructions.

6 Click OK to dismiss the message.

9

Maintenance

This chapter contains cleaning and maintenance procedures for the BD FACSArray bioanalyzer.

The following topics are covered in this chapter:

- Cleaning Procedures on page 298
- Maintenance Procedures on page 306

Cleaning Procedures

To maintain peak performance of your BD FACSArray bioanalyzer, you must keep the fluidics lines clean. Perform daily cleaning at the end of each day or after running dyes, such as propidium iodide (PI). On a monthly basis, perform the more extensive monthly cleaning procedure.

Materials

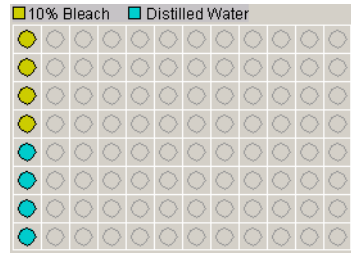
- 96-well plate
- 10% bleach solution (such as undiluted BD FACSClean™ or one part bleach with nine parts distilled [DI] water)
- DI water

Daily Cleaning

Follow this procedure at the end of every day and after running dyes, such as PI, to clean the fluidics lines of the bioanalyzer. This procedure will take less than 5 minutes.

1 Fill the following wells of a 96-well plate with the corresponding liquid.

- A1, B1, C1, and D1 with a 10% bleach solution
- E1, F1, G1, and H1 with DI water

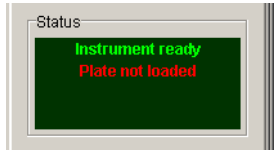


2 If an experiment is open in the Browser, close it.

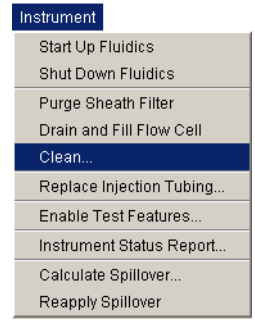
⚠ **NOTICE** If an experiment is open in the Browser, the Clean command in the Instrument menu (step 5 on page 299) will not be enabled.

3 From the Prepare workspace, click the Acquire tab in the Plate Editor.

4 Verify that the Status box shows the message *Instrument ready*.



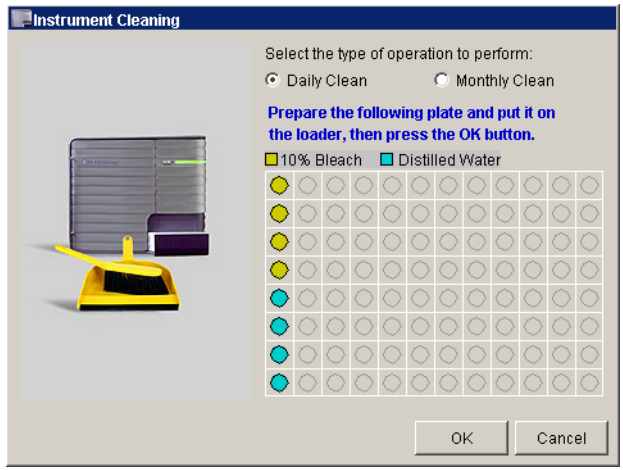
5 Choose Instrument > Clean.



For the Clean command to be enabled in the Instrument menu, the message in the Status box must read *Instrument ready*.

The Instrument Cleaning dialog appears.

6 Click the Daily Clean radio button.



The plate holder is ejected from the plate sampler.

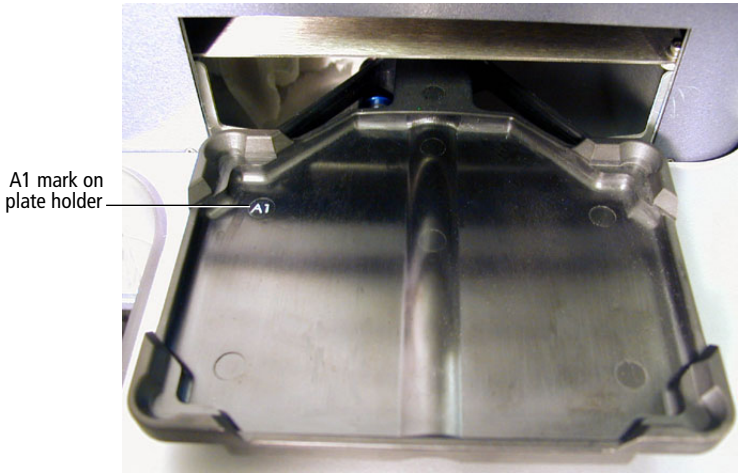
7 Put the plate on the plate holder.

⚠ **CAUTION** Movement of mechanical parts within the instrument can pinch or injure your hands or fingers. To prevent injury by moving parts, follow these precautions.

- Keep your hands away from the plate holder once you position the plate on it. The plate holder will automatically retract when you click Load.
- Keep your hands away from the plate door during instrument operation.

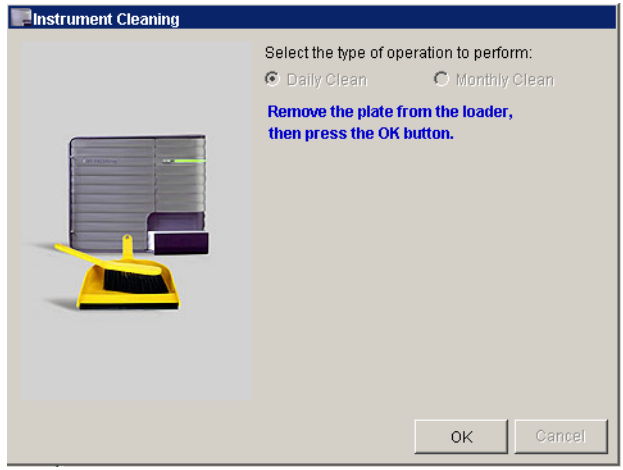
⚠ **NOTICE** To prevent damage to the bioanalyzer, remove any lid, film, or cover from the plate before loading onto the plate holder. Some combinations of plate and lid might not retract properly into the instrument, potentially causing damage to the plate and the plate sampler. Leaving a lid, film, or cover on the plate might cause clogs in the probe and sample line, affecting performance and possibly damaging the bioanalyzer.

Position the plate so that well A1 is over the A1 mark on the plate holder.



8 Click OK in the Instrument Cleaning dialog box.

- The plate sampler verifies that there is no lid on the plate, and loads the plate into the bioanalyzer.
- The bioanalyzer begins to aspirate liquid from the first well on the plate.
- The Instrument Cleaning dialog shows the progress of the cleaning cycle.
  - Wells that are being run are colored with a blue background.
  - Wells that have been run are colored white.
- When the cleaning cycle is complete, a message appears in the Instrument Cleaning dialog.



9 Remove the plate from the bioanalyzer.

10 Click OK to complete the procedure.

Monthly Cleaning

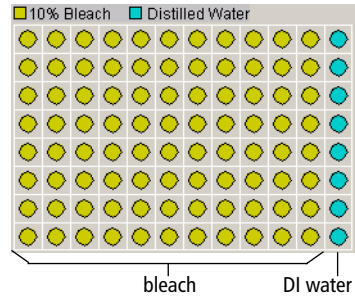
Follow this procedure on a monthly basis to clean out the bioanalyzer's fluidics lines and optical cuvette. This procedure will take less than 40 minutes.

⚠ **NOTICE** To ensure proper cleaning, it is essential to fill the wells exactly as indicated. The automated cleaning sequence first drains the optical cuvette, and then fills it with bleach from the wells in the plate. After the bleach, the optical cuvette is drained and then filled and rinsed with the remaining wells of DI water.

⚠ **NOTICE** To avoid damage to the bioanalyzer, do not put bleach into the sheath tank.

1. Fill the following wells of a 96-well plate with the corresponding liquid.

   - A1–A11, B1–B11, C1–C11, D1–D11, E1–E11, F1–F11, G1–G11, and H1–H11 with a 10% bleach solution
   - A12, B12, C12, D12, E12, F12, G12, and H12 with DI water

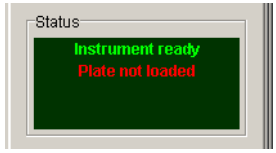


2. If an experiment is open in the Browser, close it.

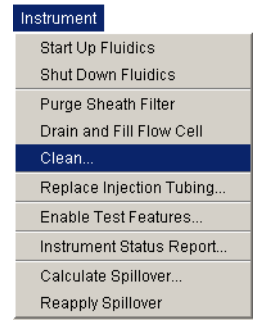
   ⚠ **NOTICE** If an experiment is open in the Browser, the Clean command in the Instrument menu (step 5 on page 303) will not be enabled.

3. From the Prepare workspace, click the Acquire tab in the Plate Editor.

4. Verify that the Status box shows the message *Instrument ready*.



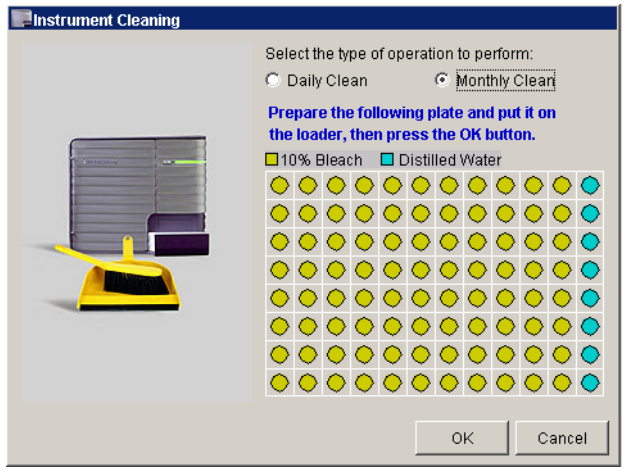
5 Choose Instrument > Clean.



For the Clean command to be enabled in the Instrument menu, the message in the Status box must read *Instrument ready.*

The Instrument Cleaning dialog appears.

6 Click the Monthly Clean radio button.



The plate holder is ejected from the plate sampler.

7 Put the plate on the plate holder.

⚠ **CAUTION** Movement of mechanical parts within the instrument can pinch or injure your hands or fingers. To prevent injury by moving parts, follow these precautions.

- Keep your hands away from the plate holder once you position the plate on it. The plate holder will automatically retract when you click Load.
- Keep your hands away from the plate door during instrument operation.

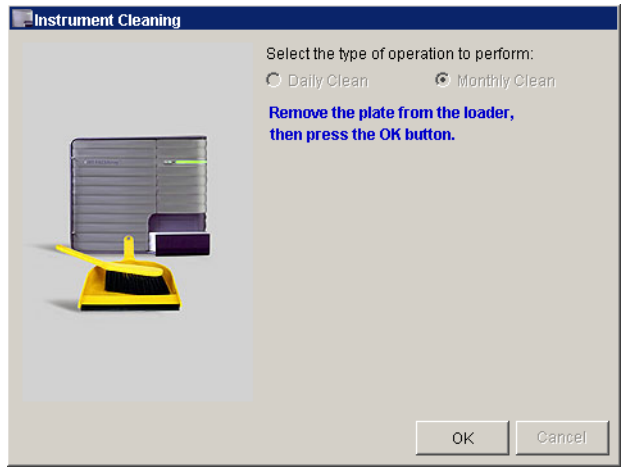
⚠ **NOTICE** To prevent damage to the bioanalyzer, remove any lid, film, or cover from the plate before loading onto the plate holder. Some combinations of plate and lid might not retract properly into the instrument, potentially causing damage to the plate and the plate sampler. Leaving a lid, film, or cover on the plate might cause clogs in the probe and sample line, affecting performance and possibly damaging the bioanalyzer.

Position the plate so that well A1 is over the A1 mark on the plate holder.

8 Click OK.

- The plate sampler verifies that there is no lid on the plate, and loads the plate into the bioanalyzer.
- The bioanalyzer begins to aspirate liquid from the first well on the plate.
- The Instrument Cleaning dialog shows the progress of the cleaning cycle.
  - Wells that are being run are colored with a blue background.
  - Wells that have been run are colored white.

- When the cleaning cycle is complete, a message appears in the Instrument Cleaning dialog.



9 Remove the plate from the bioanalyzer.

10 Click OK to complete the procedure.

Maintenance Procedures

WARNING All biological specimens and materials coming into contact with them can transmit potentially fatal disease. To prevent exposure to biohazardous agents, handle specimens and materials as if capable of transmitting infection. Dispose of waste using proper precautions and in accordance with local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

WARNING To avoid personal injury or damage to the instrument, do not perform maintenance procedures while the bioanalyzer is in Setup or Acquire mode.

This section provides maintenance instructions for your BD FACSArray bioanalyzer. Replace the specified part according to the following schedule. See Appendix B on page 357 for part numbers and ordering information

NOTICE Replacement frequency will depend on bioanalyzer usage.

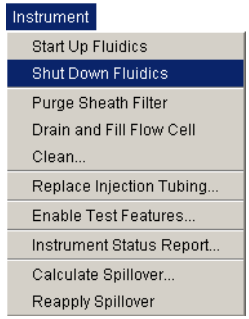
Part	Recommended Replacement Frequency	Procedure Location
fluid leaks inspection	monthly or as needed	page 307
25-mm air filter	every 12 months or as needed	page 313
75-mm air filters	every 12 months or as needed	page 314
sheath filter assembly	every 6 months or as needed	page 316
injection port tubing assembly	every 6 months or as needed	page 320
syringe	every 6 months or as needed	page 330

Leak Detection

Perform this procedure on a monthly basis.

Checking for Fluid Leaks

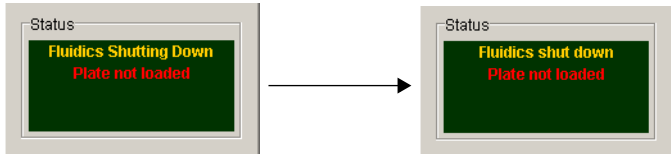
1 Choose Instrument > Shutdown Fluidics.



2 In the Prepare workspace, click the Acquire tab in the Plate Editor.

3 Monitor the Status box to verify fluidics shutdown is complete.

The Status box displays the message *Fluidics Shutting Down*, followed by *Fluidics shut down* when fluidics shutdown is complete.



4 Turn off the power to the bioanalyzer by pressing the power button on the upper front panel.

5 Open the front compartment door of the bioanalyzer.

Press, and then release the middle front portion of the front compartment door to open it.

6 Locate the locking screw.

7 Use a Phillips screwdriver to turn the screw one-quarter turn to the left.

This unlocks the grate. See Figure 9-1 on page 309.

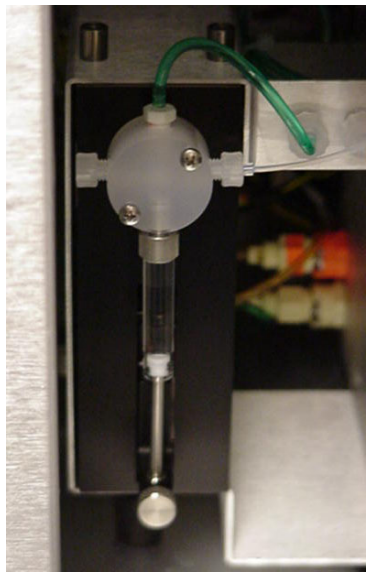
⚠ **CAUTION** If you turn the screw more than one-quarter turn to the left, the locking tab will fall out and you will no longer be able to lock the grate.

Figure 9-1 Unlocking the grate

8 Remove the grate by pivoting it toward you and pulling it up.

9 Inspect the syringe and the area beneath the syringe for signs of fluid leaks.

Check for fluid and salt crystals.



10 Hold a paper towel against the bottom of the syringe and then check for fluid on the paper towel.

If you find a fluid leak, or if you detect salt crystals, the syringe should be replaced. Follow the instructions in Syringe Replacement on page 330.

If you do not find a fluid leak or detect salt crystals, proceed to Replacing the Grate on page 310.

Replacing the Grate

1 Put the grate back into place, lining up the hole for the locking screw.

2 Replace the locking screw and turn it one-quarter turn to the right to lock it in place.

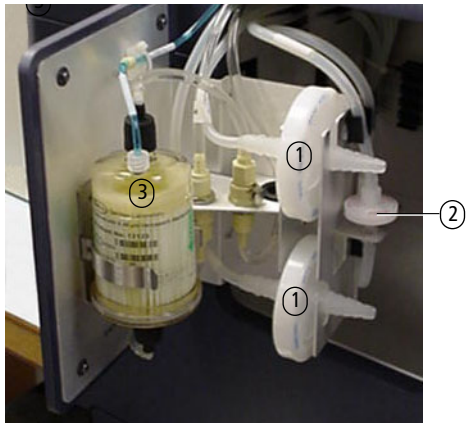
3 Close the front compartment door of the bioanalyzer.

4 Turn on the bioanalyzer, if needed.

Filter Replacement

There are four particulate filters in the bioanalyzer—one sheath filter and three air filters.

- sheath filter—prevents particulates that might be in the sheath fluid from clogging the optical cuvette or interfering with optical measurements.
- air filters—prevent dust from entering the fluidics system and filter vented air



1 75-mm air filter

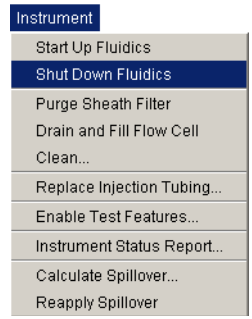
2 25-mm air filter

3 sheath filter

Preparing for Removal

Follow the instructions in this section first, and then proceed to the appropriate filter replacement procedure.

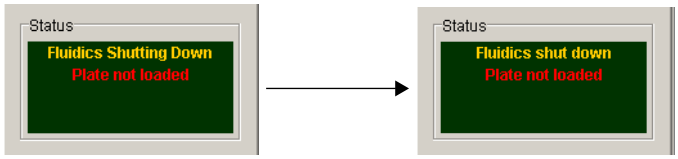
1 Choose Instrument > Shutdown Fluidics.



2 In the Prepare workspace, click the Acquire tab in the Plate Editor.

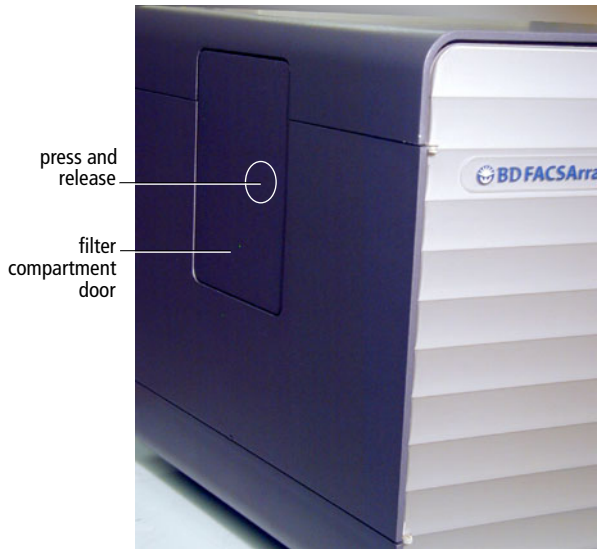
3 Monitor the Status box to verify fluidics shutdown is complete.

The Status box displays the message *Fluidics Shutting Down,* followed by *Fluidics shut down* when fluidics shutdown is complete.



4 Open the filter compartment door.

Press and then release the indented portion of the filter compartment door to open it.



5 Proceed to the appropriate filter replacement procedure.

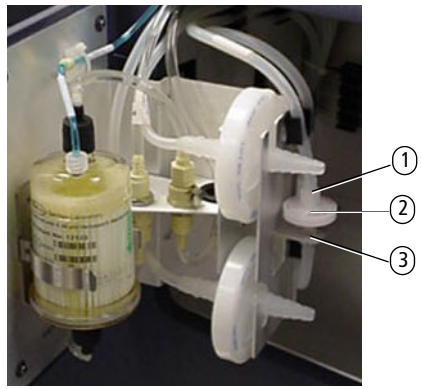
- Replacing the 25-mm Air Filter on page 313
- Replacing the 75-mm Air Filters on page 314
- Replacing the Sheath Filter Assembly on page 316

Replacing the 25-mm Air Filter

The consumables kit shipped with your bioanalyzer contains two replacement 25-mm air filters. See page 357 for part numbers and ordering information.

1 If you haven't already done so, follow the instructions for Preparing for Removal on page 311.

2 Locate the 25-mm air filter.



1 Luer connector

2 25-mm air filter

3 bracket fitting

3 Remove the 25-mm air filter.

- Unscrew the top Luer connector from the air filter by twisting counterclockwise.
- Pull the air filter up from the bracket fitting.

☑ **Tip** If you have trouble removing the 25-mm air filter, try twisting it while pulling it up from the bracket fitting.

- Discard the air filter as biohazardous waste.

4 Connect the new 25-mm air filter.

☑ **Tip** To keep track of when the air filter was last replaced, write the date on the side of the new air filter before connecting it.

- Push the new air filter into the bracket fitting.
- Screw the top Luer connector onto the new air filter by twisting clockwise.

5 Verify that the filter is secure.

6 Close the filter compartment door.

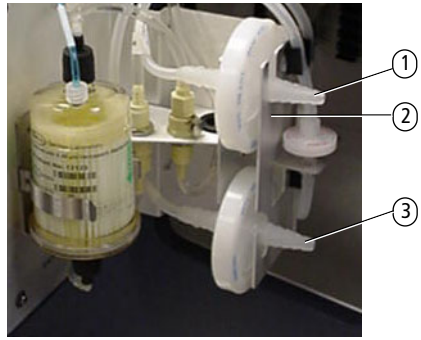
Replacing the 75-mm Air Filters

The consumables kit shipped with your bioanalyzer contains two replacement 75-mm air filters. See Appendix B on page 357 for part number and ordering information.

1 If you haven't already done so, follow the instructions for Preparing for Removal on page 311.

2 Locate the 75-mm air filters (Figure 9-2 on page 315).

Figure 9-2 Locating the 75-mm air filters



1 upper 75-mm air filter

2 bracket

3 lower 75-mm air

3 Remove the upper 75-mm air filter.

- Pull the air filter up and out of the bracket.
- Pull the tubing from barbed fitting.
- Discard the air filter as biohazardous waste

4 Connect the new 75-mm air filter.

☑ **Tip** To keep track of when the air filter was last replaced, write the date on the side of the new air filter before connecting it.

- Connect the tubing to the new 75-mm air filter by pushing the tubing over the barbed fitting of the filter.
- Push the air filter down into the bracket.

5 Repeat step 3 and step 4 to replace the lower 75-mm air filter.

6 Close the filter compartment door.

Replacing the Sheath Filter Assembly

⚠ **CAUTION** Because the sheath filter is pressurized when the bioanalyzer is on, BD recommends changing the sheath filter assembly after the instrument has been shut off for at least one hour.

The consumables kit shipped with your bioanalyzer contains one replacement sheath filter. See Appendix B on page 357 for part number and ordering information.

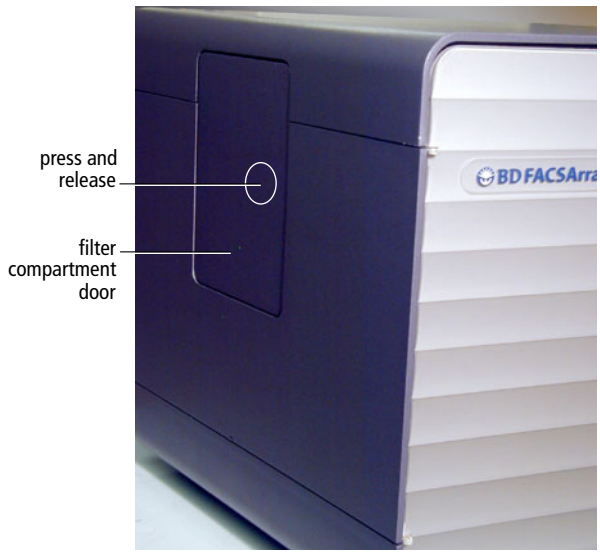
Disconnecting the Sheath Filter Assembly

1. If you haven't already done so, perform step 1 through step 3 in Preparing for Removal on page 311.

2. Turn off the power to the bioanalyzer by pressing the power button on the upper front panel.

3. Open the filter compartment door.

   Press and then release the indented portion of the filter compartment door to open it.



4 Locate the sheath filter assembly.

Figure 9-3 sheath filter assembly

① male connector from filter top

② female quick disconnect for male connector (①) from filter top

③ female quick disconnect for male connector (③) from filter bottom

④ male connector from filter bottom

⑤ Luer connector

⑥ sheath filter

⑦ sheath filter bracket

5 Place paper towels on the workspace underneath the sheath filter assembly to catch drips during replacement.

⚠ **CAUTION** In case there is residual pressure in the fluidic lines, firmly grip the male connectors before pressing the female quick disconnects and keep the connectors pointing away from you. Cover the quick disconnects with a paper towel to catch any fluid leaks. Wear suitable protective clothing, eyewear, and gloves.

6 While holding the male connector (①) from the filter top, press the female quick disconnect (②) to release the male connector (Figure 9-3).

See Figure 9-4 on page 319 for a close up view of the connector locations.

7 While holding the male connector (③) from the filter bottom, press the female quick disconnect (④) to release the male connector (Figure 9-3).

See Figure 9-4 on page 319 for a close up view of the receptacle location.

8 Unscrew and remove the Luer connector (⑤) (Figure 9-3).

Unscrew the Luer connector by twisting counterclockwise.

9 Remove the sheath filter assembly by pulling it out of the sheath filter bracket.

10 Discard the sheath filter assembly as biohazardous waste.

Connecting the Replacement Sheath Filter Assembly

1 Grasp the new sheath filter assembly and orient it so that the green arrow on the side of the filter is pointing down (Figure 9-4 on page 319).

2 Push the new sheath filter assembly into the sheath filter bracket.

☑ **Tip** To keep track of when the sheath filter was last replaced, write the date on the side of the new sheath filter before connecting it.

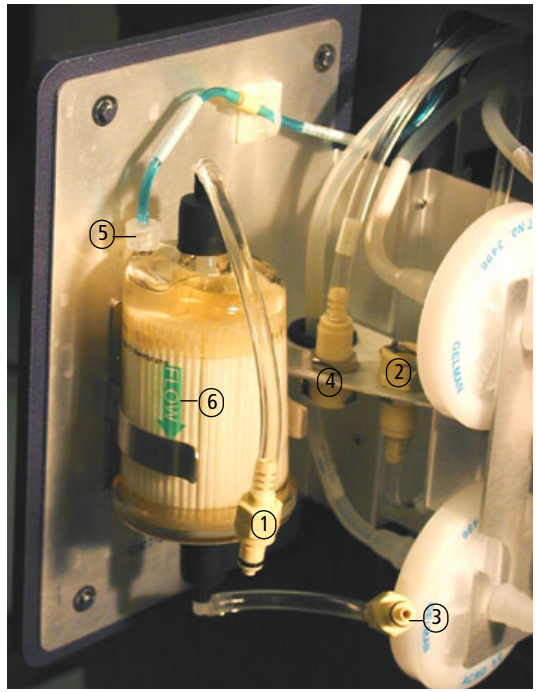
3 Attach the Luer connector (⑤) by twisting clockwise (Figure 9-4 on page 319).

⚠ **NOTICE** If not firmly connected, sheath fluid can leak out of this connection at a rapid rate.

4 Attach the top male and bottom female sheath connectors (Figure 9-4 on page 319).

- Gently push the male connector (①) from the filter top into the female quick disconnect (②) until it snaps into place.
- Gently push the male connector (③) from the filter bottom into the female quick disconnect (④) until it snaps into place.

Figure 9-4 Reconnecting the sheath filter



1. male connector from filter top
2. female quick disconnect for male connector (1) from filter top
3. male connector from filter bottom
4. female quick disconnect for male connector (3) from filter bottom
5. Luer connector
6. green arrow

5 Turn on the power to the bioanalyzer by pressing the power button on the upper front panel.

6 Choose Instrument > Purge Sheath Filter.

7 Observe the sheath filter to ensure it fills with sheath fluid.

The sheath filter will not fill immediately, but should start to fill within 2 minutes.

If the sheath filter does not fill, verify that the disconnects are secure and there is no leakage around the fittings. See Troubleshooting on page 337 for more information.

8 Close the filter compartment door.

Injection Port Tubing Assembly Replacement

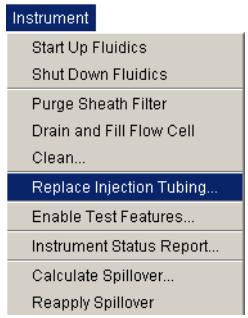
The consumables kit shipped with your bioanalyzer contains one replacement injection port tubing assembly. See Appendix B on page 357 for part number and ordering information.

Replace the injection port tubing assembly every 6 months or as needed. If you observe any of the following symptoms, immediately replace the injection port tubing assembly.

- leaking around the connectors or along the sample tubing*
- a gradual degradation of bioanalyzer performance

Preparing for Removal

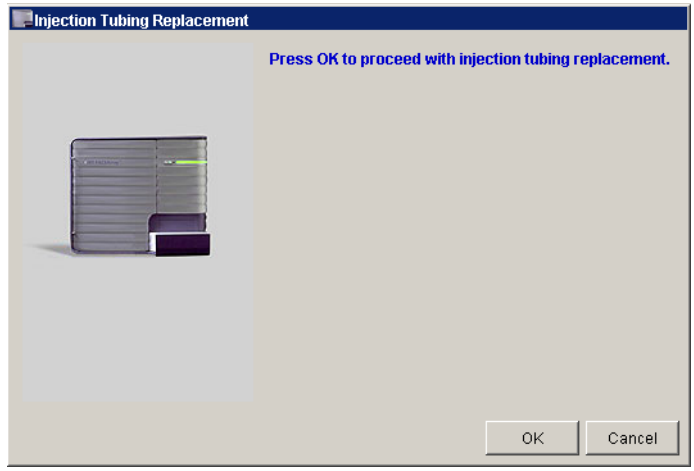
1 Choose Instrument > Replace Injection Tubing.



The following dialog box appears (Figure 9-5 on page 321).

* You must remove the injection port to detect leaking around the injection port tubing connectors or along the sample tubing.

Figure 9-5 Injection Probe Tubing Removal dialog box



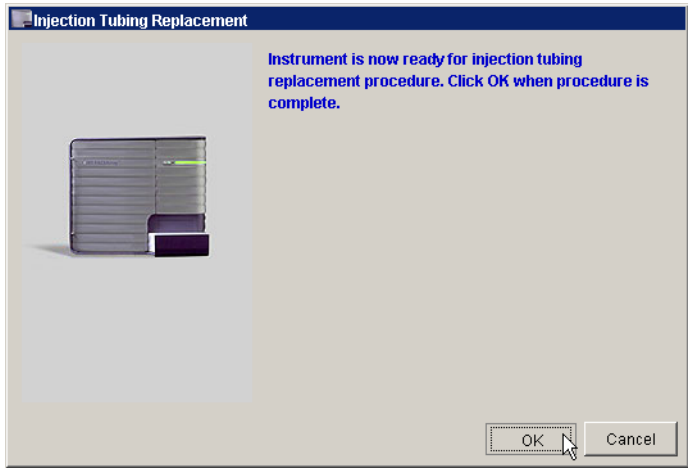
2 Click OK to shut down the bioanalyzer fluidics.

Messages appear in the Injection Tubing Removal dialog indicating the following:

- fluidics are being shut down
- injection probe is being moved to a safe position

Once the probe is in the safe position, the following message appears in the Injection Tubing Removal dialog (Figure 9-6 on page 322).

Figure 9-6 Ready to replace injection port tubing dialog


Injection Tubing Replacement
Instrument is now ready for injection tubing replacement procedure. Click OK when procedure is complete.
OK
Cancel

Removing the Grate

WARNING Do not perform this procedure until the fluidics shutdown is complete, the probe is in the safe position, and the message shown in Figure 9-6 appears in the Injection Tubing Removal dialog.

1. Open the front compartment door of the bioanalyzer.

   Press and then release the middle front portion of the front compartment door to open it.

2. Locate the locking screw (Figure 9-7 on page 323).

Figure 9-7 Area under front compartment door

3 Use a Phillips screwdriver to turn the screw one-quarter turn to the left (Figure 9-8 on page 324).

This unlocks the grate.

⚠ **CAUTION** If you turn the screw more than one-quarter turn to the left, the locking tab will fall out and you will no longer be able to lock the grate.

Figure 9-8 Unlocking the grate

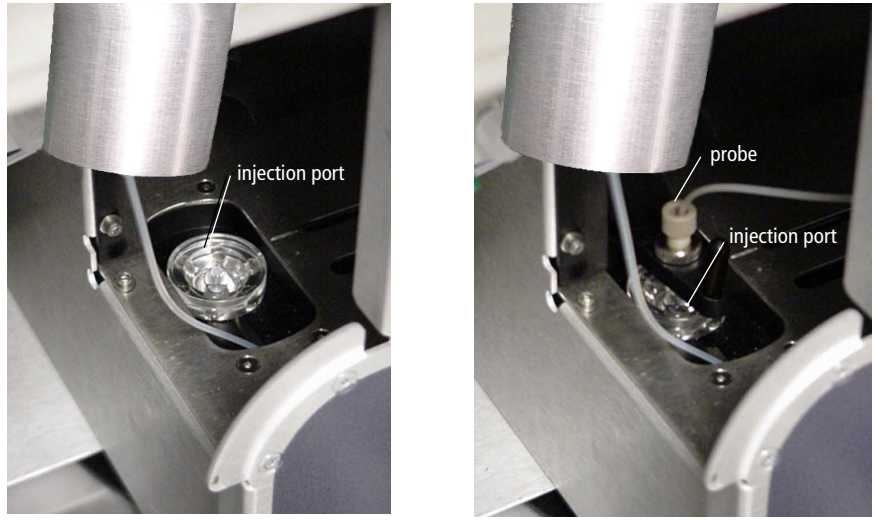
4 Remove the grate by pivoting it toward you and pulling it up.

Removing the Injection Port and Tubing Assembly

1 Verify that the probe is **not** in the injection port (Figure 9-9 on page 325).

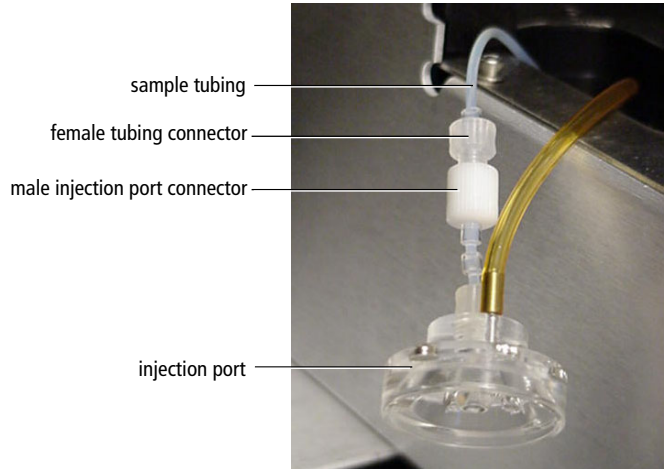
⚠ **CAUTION** If the probe is still in the injection port (incorrect position), replace the grate and see Instrument Troubleshooting on page 338 for instructions.

Figure 9-9 Probe in the correct (left) and incorrect (right) position for removal

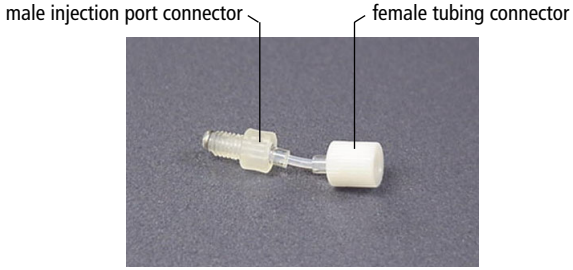


2 Grasp the injection port and turn it slightly until it disengages from the base plate.

3 Gently pull the injection port up and out of its housing until the connectors and tubing are exposed.



4 Unscrew the male injection port connector and the female tubing connector to remove the tubing from the injection port.



5 Discard the injection port tubing as biohazardous waste.

Installing New Injection Port Tubing Assembly

⚠ **CAUTION** Do not let the sample tubing fall into the bioanalyzer. If it does, do not attempt to retrieve it. To prevent personal injury, call Technical Support for assistance.

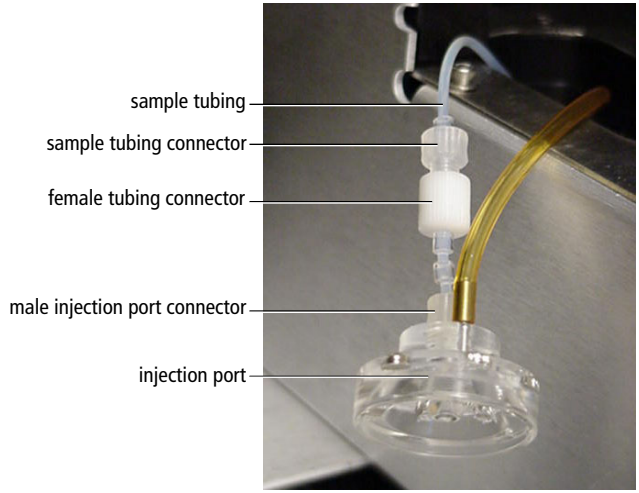
☑ **Tip** Before installing the injection port tubing assembly, watch the training CD showing installation.

1 Connect the new injection port tubing assembly (Figure 9-10 on page 327).

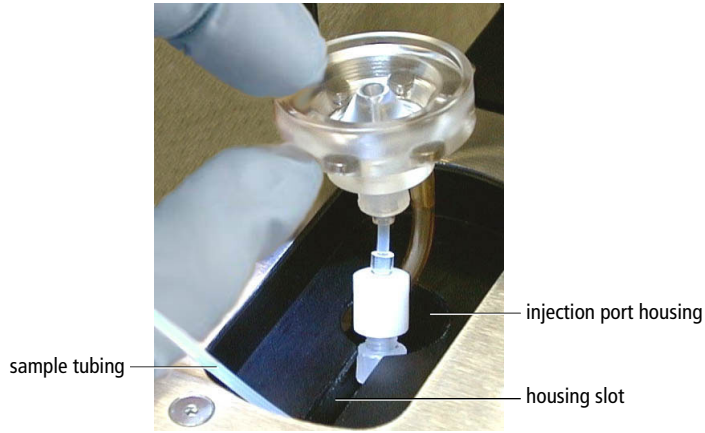
- Screw the female tubing connector onto the sample tubing connector of the bioanalyzer.
- Screw the male injection port connector onto the injection port.

⚠ **NOTICE** Verify that the connectors are on tight.

Figure 9-10 Connecting the new injection port tubing assembly



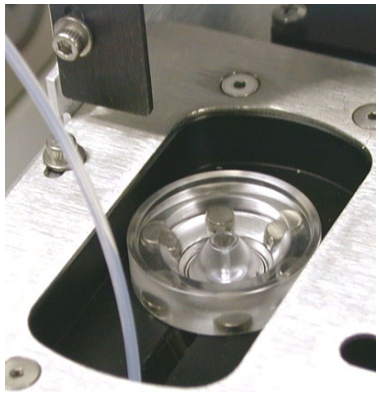
2 Verify that the sample tubing is routed through the housing slot.



3 Gently push the tubing and injection port back into the housing on the bioanalyzer.

Twist the injection port while pushing it into the housing to ensure that it is level and snaps into place.

Figure 9-11 Replacing the tubing and injection port



Replacing the Grate

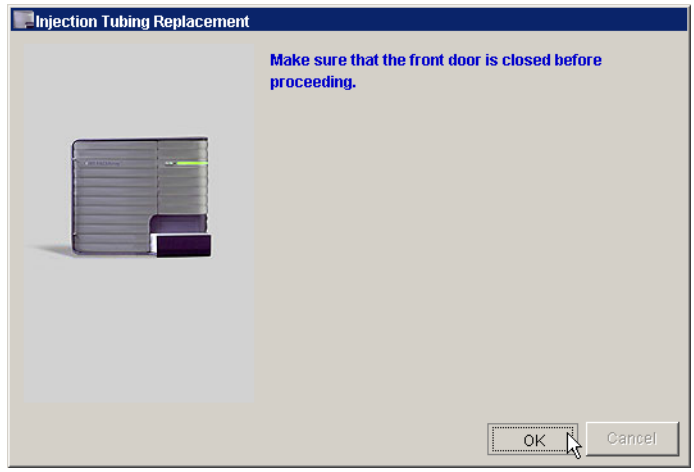
1 Put the grate back into place, lining up the hole for the locking screw.

2 Replace the locking screw and turn it one-quarter turn to the right to lock it in place.

3 Close the front compartment door of the bioanalyzer.

4 Click OK (Figure 9-12 on page 329).

Figure 9-12 Reminder dialog



5 Click OK to exit the dialog and complete the procedure.

Verifying Performance

1 Run a known sample on the bioanalyzer.

You can use QC beads to check performance.

2 Verify that the event rate is at an expected level for the concentration and flow rate used.

3 If the event rate is lower than expected, follow the instructions in Removing the Grate on page 322.

4 Verify the new injection port tubing connectors are on tight.

5 If the problem persists, call BD Biosciences.

Syringe Replacement

The consumables kit shipped with your bioanalyzer contains one replacement syringe. See Appendix B on page 357 for part number and ordering information.

⚠ **WARNING** To avoid personal injury or damage to the instrument, turn off the power to the bioanalyzer before performing this procedure.

Shutting Down the Bioanalyzer

1. Choose Instrument > Shutdown Fluidics.
2. Turn off the power to the bioanalyzer by pressing the power button on the upper front panel.

Removing the Grate

1. Open the front compartment door of the bioanalyzer.

   Press and then release the middle front portion of the front compartment door to open it.
2. Locate the locking screw (Figure 9-13 on page 331).

Figure 9-13 Locating the locking screw

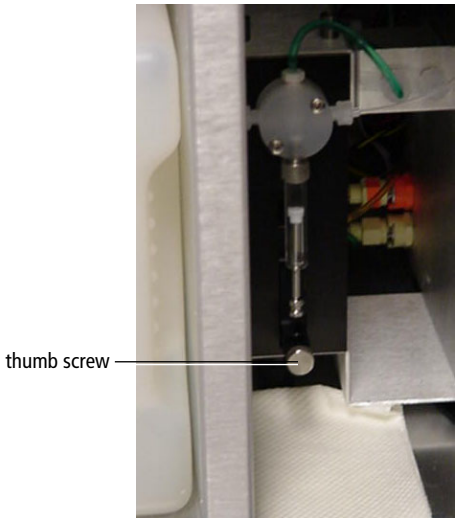
3 Use a Phillips screwdriver to turn the locking screw one-quarter turn to the left to unlock the grate.

⚠ **CAUTION** If you turn the screw more than one-quarter turn to the left, the locking tab will fall out and you will no longer be able to lock the grate.

4 Remove the grate by pivoting it toward you and pulling it up.

Removing the Syringe

1 Place a paper towel under the syringe to absorb drips when the syringe is removed.



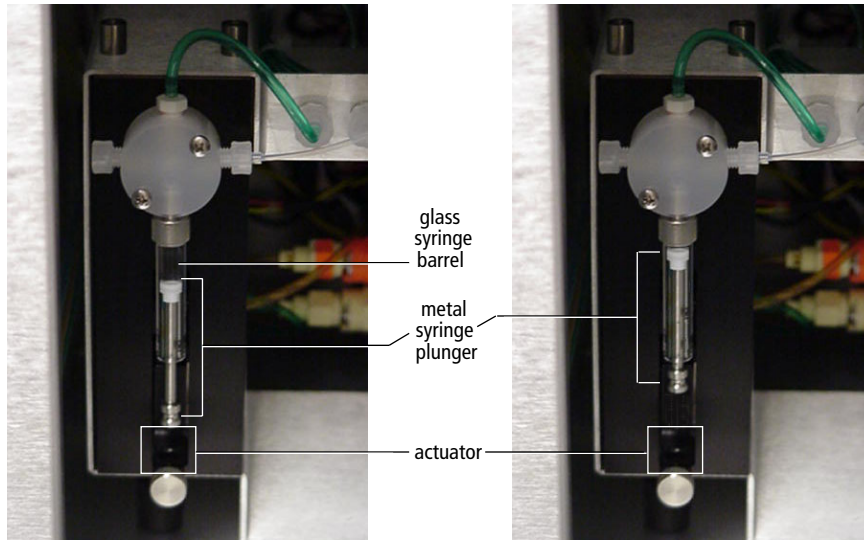
2 Loosen, but do not remove, the thumb screw at the bottom of the syringe assembly.

Turn the thumb screw counterclockwise to loosen it.

3 Grip the thumb screw and push the actuator down until it stops. (Figure 9-14 on page 333).

4 Grip the metal plunger of the syringe and gently push it up until it stops.

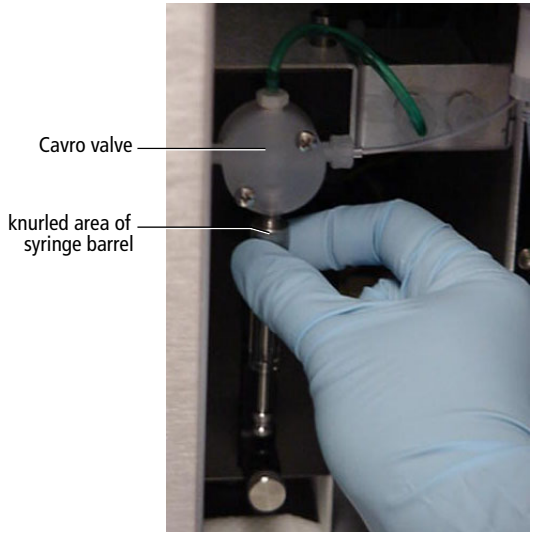
Figure 9-14 Metal syringe plunger down (left) and all the way up (right)



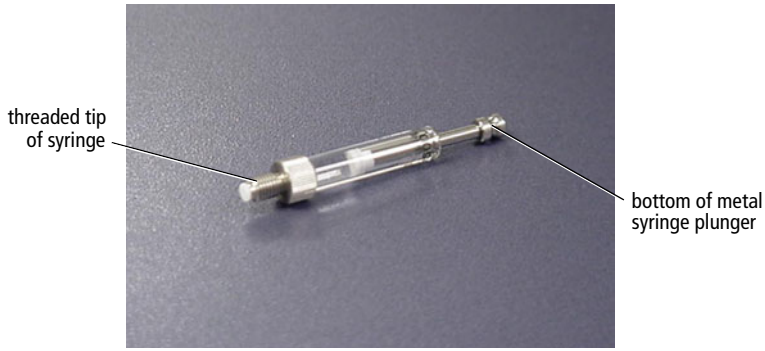
5 Turn the knurled area at the top of the syringe barrel counterclockwise to loosen it (Figure 9-15 on page 334).

⚠ **CAUTION** To avoid personal injury, handle the syringe carefully. The syringe barrel is made of glass.

Figure 9-15 Loosening the syringe barrel



6 Remove the syringe from the Cavro valve.



7 Discard the syringe in a biohazard sharps container.

Installing the New Syringe

1. Insert the threaded tip of the new syringe into the Cavro valve and tighten the syringe barrel.

   Turn the knurled area at the top of the syringe barrel clockwise to tighten it. Verify the barrel is on tight.

2. Pull the metal syringe plunger down until it is seated at the bottom of the actuator.

3. Tighten the thumb screw attached to the actuator.

4. Remove the paper towel from the syringe area.

Replacing the Grate

1. Put the grate back into place, lining up the hole for the locking screw.

2. Replace the locking screw and turn it one-quarter turn to the right to lock it in place.

3. Close the front panel of the bioanalyzer.

4. Turn on the bioanalyzer, if needed.

10

Troubleshooting

The tips in this section are designed to help you troubleshoot your experiments. If additional assistance is required, contact your local BD Biosciences technical support representative. Refer to our website, http://www.bdfacs.com, for up-to-date contact information.

Troubleshooting suggestions can be found under the following topics.

- Instrument Troubleshooting on page 338
- Software Troubleshooting on page 348
- Analysis Troubleshooting on page 349
- Error Messages on page 350
- LED Indicator Status on page 352

WARNING All biological specimens and materials coming into contact with them can transmit potentially fatal disease. To prevent exposure to biohazardous agents, handle all biological specimens and materials as if capable of transmitting infection. Dispose of waste using proper precautions and in accordance with local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

Instrument Troubleshooting

Observation	Possible Causes	Recommended Solutions
Acquisition Status frame ( ) shows threshold rate, threshold events, and processed events, but no events appear on plots	Detector voltages too high or too low	Adjust detector voltages.
	Plots are gated on a population that has no events	**1** Choose Populations > Show Populations and verify that the appropriate population is selected. **2** Determine if gate encloses the appropriate population.
	No color assigned to population	Verify that a color for the population is selected in the Population Hierarchy view.
Acquisition Status frame ( ) shows no threshold or processed events	Threshold too high/detector voltages too low	Reduce threshold and/or increase detector voltages.
	No sample in selected well	Select appropriate well containing sample.
		Add sample to well.
	Air bubble or debris in fluidics line	Choose Instrument > Drain and Fill Flow Cell.
	Air bubble in flow cell	Choose Instrument > Drain and Fill Flow Cell.
		Verify that you are using BD FACS sheath solution with surfactant.

Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Acquisition Status frame ( ) shows no threshold or processed events (cont)	Clog in sample line	**1** Stop acquisition. **2** Follow instructions in Preparing for Removal on page 320 through page 322. **3** Follow instructions for removing the grate on page 322 through page 324. **4** Observe probe, syringe, and sample tubing. **5** If you observe a fluid leak, call BD Biosciences. **6** If you observe no leak, replace the grate following instructions on page 328. **7** Choose Instrument > Startup Fluidics. **8** Follow instructions for running daily cleaning on page 298.
	No sheath supply	Check pressure on Status tab of Instrument frame.
		Verify that connectors on sheath tank are seated properly.
		Verify that connectors on sheath filter assembly are seated properly.
	Air in sheath filter	Choose Instrument > Purge Sheath Filter.

Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Acquisition Status frame ( ) shows no threshold or processed events (cont)	Loader tubing leak	**1** Stop acquisition. **2** Follow instructions in Preparing for Removal on page 320 through page 322. **3** Follow instructions for removing the grate on page 322 through page 324. **4** Observe probe, syringe, and sample tubing. **5** If injection port tubing assembly was recently replaced, remove it by following the procedure in Removing the Injection Port and Tubing Assembly on page 324. **6** Check for leaks at the fittings. **7** Tighten fittings. **8** If leak persists, follow instructions given in Installing New Injection Port Tubing Assembly on page 326.
	Probe not sealing in injection port	**1** Stop acquisition. **2** Follow instructions in Preparing for Removal on page 320 through page 322. **3** Follow instructions for removing the grate on page 322 through page 324. **4** Observe probe and injection port. **5** If bubbles appear around the probe, call BD Biosciences.

Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Acquisition Status frame ( ) shows no threshold or processed events (cont)	Probe misses injection port	**1** Stop acquisition. **2** Follow instructions in Preparing for Removal on page 320 through page 322. **3** Follow instructions for removing the grate on page 322 through page 324. **4** Observe probe. **5** If probe is not seated in injection port, call BD Biosciences.
Acquisition Status frame ( ) shows a lower than expected event rate	Sample too dilute	Concentrate sample.
	Sample not well mixed	Mix sample.
		Adjust mix settings. See Inspector on page 103 and Understanding Volumes on page 105 for more information.
	Partial clog	Run daily cleaning cycle by choosing Instrument > Clean > Daily Clean.
		Replace Injection port tubing. See Injection Port Tubing Assembly Replacement on page 320.
	Threshold too high/threshold parameter voltage too low	Decrease threshold and/or increase threshold parameter voltage.

Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Acquisition Status frame ( ) shows a lower than expected event rate (cont)	Injection port leaks	**1** Stop acquisition. **2** Follow instructions in Preparing for Removal on page 320 through page 322. **3** Follow instructions for removing the grate on page 322 through page 324. **4** Follow instructions for Removing the Injection Port and Tubing Assembly on page 324 through page 326. **5** Inspect fittings and sample tubing. **6** If you detect a fluid leak in the injection port tubing or fittings, follow instructions given in Installing New Injection Port Tubing Assembly on page 326 through page 328. **7** Call BD Biosciences if you suspect there is a problem with the injection port seal.

Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Acquisition Status frame ( ) shows a lower than expected event rate (cont)	Air in syringe	**1** Stop acquisition. **2** Follow instructions for shutting down the bioanalyzer on page 330. **3** Follow instructions for removing the grate on page 330 through page 332. **4** Observe syringe. **5** If you see an air bubble at the top of the syringe, replace the grate following instructions on page 335. **6** Choose Instrument > Startup Fluidics.
Acquisition Status frame ( ) shows an erratic event rate	Sample volume set too high—aspirated air with sample	Adjust sample volume. See Inspector on page 103 and Understanding Volumes on page 105. ⚠ **NOTICE** Remember to account for plate dead volume and overhead volume (20 µL).
	Bubbles in sample due to incorrect mixing parameters	Adjust mixing volume to avoid aspirating air. See Inspector on page 103 and Understanding Volumes on page 105. ⚠ **NOTICE** Remember to account for plate dead volume when setting mixing volume.

Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Acquisition Status frame ( ) shows an erratic event rate (cont)	Injection port leaks	**1** Stop acquisition. **2** Follow instructions in Preparing for Removal on page 320 through page 322. **3** Follow instructions for removing the grate on page 322 through page 324. **4** Follow instructions for Removing the Injection Port and Tubing Assembly on page 324 through page 326. **5** Inspect fittings and sample tubing. **6** If you detect a fluid leak in the injection port tubing or fittings, follow instructions given in Installing New Injection Port Tubing Assembly on page 326 through page 328. **7** Call BD Biosciences if you suspect there is a problem with the injection port seal.
Acquisition Status frame ( ) shows a higher than expected event rate	Sample concentration too high	Dilute sample.
	Threshold too low—capturing too many noise/debris events	Increase threshold and/or reduce detector voltages.
	Set wrong sample rate for application	Adjust sample flow rate in the Acq. tab of the Experiment Inspector. See Inspector on page 103.

Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Unexpected events in plot	Incorrect logic in Population Hierarchy	Verify the gating strategy.
	Incorrect population(s) in plot	**1** Right-click the plot and choose Show Populations. **2** Verify that the appropriate populations are displayed.
	Incorrect drawing order	Verify that the required population is not hidden by another population. Right-click the plot and choose Order Populations by Count.
Distorted light scatter pattern	Sample volume set too high—aspirated air with sample	Adjust sample volume to avoid aspirating air. See Inspector on page 103 and Understanding Volumes on page 105. ⚠ **NOTICE** Remember to account for plate dead volume and overhead (20 µL) volume.
	FSC or SSC parameters set incorrectly	Adjust detector settings.
		Ensure the Log display is selected or deselected according to application requirements.
Poor FSC sensitivity	Sample preparation problem	Check instrument performance by running QC particles
	Threshold set incorrectly	Increase FSC threshold.
	Dirty sheath filter	Replace sheath filter assembly.
	Inside walls of optical cuvette are dirty	Run monthly cleaning cycle by choosing Instrument > Clean > Monthly Clean.

Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Abnormally high CVs	Air bubble in optical cuvette or air in fluidic lines	Choose Instrument > Drain and Fill Flow Cell and/or Start Up Fluidics.
	Partial clog in sample line	Run daily cleaning cycle by choosing Instrument > Clean > Daily Clean.
	Clogged sheath filter	Replace sheath filter assembly.
	Incorrect or improperly prepared sample	Check sample preparation.
		Confirm bioanalyzer performance by running QC particles.
	Sample flow rate too high	Reduce sample flow rate.
	Acquired incorrect well	Select appropriate well, verify loader settings, and reacquire.
	APD voltage set too low	Adjust APD voltage.
	PMT voltage set too low	Adjust PMT voltage.
	Change in sheath pressure	Check Sheath pressure in Fluidics tab of Instrument Settings frame. If the pressure is >4 psi, replace the 25-mm and upper 75-mm air filter.
Syringe leaking	Worn syringe seal	**1** Check for fluid leaks by performing Leak Detection on page 307. **2** If you find a fluid leak or detect salt crystals, follow the procedure in Syringe Replacement on page 330.

Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Detect fluid underneath and/or in front of the bioanalyzer	Injection port tubing leak	**1** Stop acquisition. **2** Follow instructions in Preparing for Removal on page 320 through page 322. **3** Follow instructions for removing the grate on page 322 through page 324. **4** Follow instructions for Removing the Injection Port and Tubing Assembly on page 324 through page 326. **5** Inspect fittings and sample tubing. **6** If you detect a fluid leak in the injection port tubing or fittings, follow instructions given in Installing New Injection Port Tubing Assembly on page 326 through page 328. **7** Call BD Biosciences if you suspect there is a problem with the injection port seal.
	Fluidics malfunction	**1** Stop acquisition. **2** Follow instructions in Preparing for Removal on page 320 through page 322. **3** Follow instructions for removing the grate on page 322 through page 324 **4** Confirm source of leak is not injection port tubing. **5** Call BD Biosciences.
	Waste tank overflow	Call BD Biosciences.

Software Troubleshooting

Observation	Possible Causes	Recommended Solutions
Unable to quit software because software is frozen	Software processing error	**1** Access the Windows 2000 Task Manager. **2** Choose BD FACSArray system software and click End.
Unable to view a frame in the workspace	Frame obstructed from view by another frame	Reduce the size of the other frames on the workspace, starting with the Plate Editor frame.

Analysis Troubleshooting

Observation	Possible Causes	Recommended Solutions
Data not displayed on some plots	Plot gated on population that has no events	**1** Choose Populations > Show Populations. **2** Verify that an appropriate population is selected. **3** Determine whether the gate encloses the appropriate population.
	No color assigned to population	**1** Check Population Hierarchy view to verify a color is assigned to the population. **2** Choose a color for the population if none chosen.

Error Messages

Observation	Possible Causes	Recommended Solutions
Sheath buffer overflow	Temporary fluctuation trips sensor	Quit software and restart. • If it was a fluctuation, the problem will correct itself. • If problem persists, call BD Biosciences.
	Fluidics malfunction	Call BD Biosciences.
Waste buffer too high	Temporary fluctuation trips sensor	Quit software and restart. • If it was a fluctuation, the problem will correct itself. • If problem persists, call BD Biosciences.
	Fluidics malfunction	Call BD Biosciences.
Waste pressure high	Waste tank connector not plugged in or partial failure	Fully seat connector.
	Clogged vent filter	Replace lower 75-mm air filter. See Replacing the 75-mm Air Filters on page 314 for instructions.
	Fluidics malfunction	Call BD Biosciences.
Instrument not connected	Improperly connected or missing ethernet cable	Check Ethernet connections.
	Incorrect or deleted IP address	Call BD Biosciences.
	Error in digital electronics initialization	**1** Turn off bioanalyzer. **2** Turn on bioanalyzer and restart software.

Error Messages (continued)

Observation	Possible Causes	Recommended Solutions
Sheath buffer time-out	Sheath tank connector not plugged in or partially plugged in	Fully seat sheath tank connector
	Sheath filter installed improperly	Check sheath filter connections.
	Fluidics malfunction	Call BD Biosciences.
Loader error	Plate improperly seated	Unload plate and reseat it.
	Plate or plate and lid jammed in the door	**1** Stop acquisition. **2** Remove plate. **3** Choose Instrument > Startup Fluidics to reinitialize the loader.
	Lid on plate	Remove plate lid.
	Injection port improperly seated	**1** Stop acquisition. **2** Follow instructions in Preparing for Removal on page 320 through page 322. **3** Follow instructions for removing the grate on page 322 through page 324. **4** Observe probe and injection port and verify probe is seated in port. **5** If the probe and injection port appear normal, follow instructions for replacing the grate on page 328. **6** Restart the bioanalyzer. **7** If problem persists, call BD Biosciences.

LED Indicator Status

LED Color	LED State	Instrument Status
Green	Unblinking	Instrument ready to acquire.
Amber	Unblinking	Instrument not ready to acquire.
Amber	Blinking	Fluidics tanks need attention.
Red	Unblinking	System error. Check the Status tab of the Instrument frame for status messages.

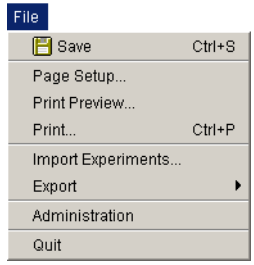
Appendix A

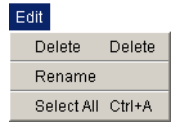
Menus and Keyboard Shortcuts

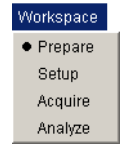
This appendix provides a visual map of all BD FACSArray software application menus and a list of the available keyboard shortcuts. For more information, see the following:

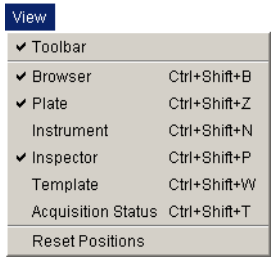
- Application Menus on page 354
- Contextual Menus on page 355
- Keyboard Shortcuts on page 356

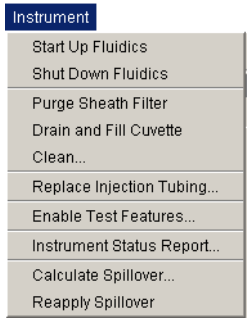
Application Menus

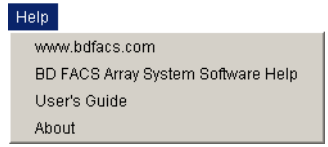










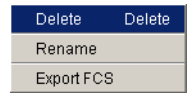


Contextual Menus

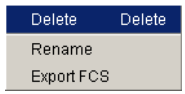
Experiment

Plate

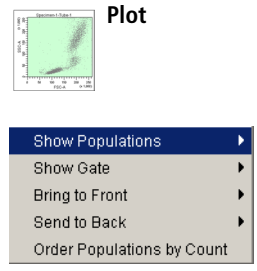
Sample



Well

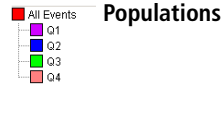


Plot

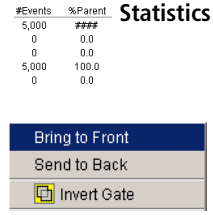


Parameters

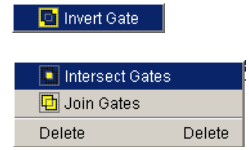
Populations



Statistics



Gate



Keyboard Shortcuts

Keyboard shortcuts are provided for the following functions.

Objective	Key Combination	Condition to Activate Shortcut
New Experiment	Ctrl-E	
Save	Ctrl-S	Experiment must be open
Open Experiment	Ctrl-O	Closed Experiment must be selected
Close Experiment	Ctrl-W	Open Experiment must be selected
Print	Ctrl-P	Experiment must be open
New Plate	Ctrl-Y	Experiment must be open
Delete	Delete	Item must be selected
Select All	Ctrl-A	Template must be selected

Keyboard shortcuts are provided to show or hide the following frames.

Frame	Key Combination
Browser	Ctrl-Shift-B
Plate	Ctrl-Shift-Z
Instrument Status	Ctrl-Shift-N
Inspector	Ctrl-Shift-P
Template	Ctrl-Shift-W
Acquisition Status	Ctrl-Shift-T

Appendix B

Specifications and Consumables

This appendix contains the instrument specifications and consumables list. For more information, see the following:

- Instrument Specifications on page 358
- Consumables on page 359

Instrument Specifications

Space Requirements

- Height: 19.6 in. (49.8 cm)
- Width: 22.1 in. (56.1 cm)
- Depth: 25.3 in. (64.3 m)
- Weight: <90 lbs (<40.8 kgs) without fluid*

Operating Conditions

- Operating temperature range: 18 to 30ºC
- Storage temperature range: 0 to 45ºC
- Humidity range: 20 to 80% relative humidity, non-condensing
- Altitude: 0 to 7000 feet

Electrical

- 100–250 VAC, 50/60 Hz
- 15 Amp

* The weight of the BD FACSArray does not include the weight of peripherals or full waste and sheath tanks.

Consumables

The following table lists catalog numbers for consumables that are included in the Consumables kit shipped with the BD FACSArray bioanalyzer. The table also includes the description and catalog numbers of 96-well plates that are compatible for use on the bioanalyzer.

Part	Catalog Number
25-mm hydrophobic filter[a]	343538
75-mm hydrophobic filter[a]	336622
sheath filter assembly[a]	336619
injection port tubing assembly[a]	336635
500 µL syringe assembly[a]	335239
cap for 4 L sheath tank[a]	336904
20 L cube of BD FACS Sheath Solution with Surfactant[a]	336524 (US) 336911 (Europe)
5 L BD FACSClean Solution[a]	340345
BD Falcon 96-well polystyrene U-bottom plate[a]	353910 (without lid)
BD Falcon™ 96-well polystyrene flat-bottom plate	353915 (without lid)
BD Falcon™ 96-well polystyrene lid fits 353910 and 353915 plates	353071
BD Falcon 96-well polypropylene U-bottom plate	351190 (without lid)
BD Falcon™ 96-well polypropylene V-bottom plate	353263 (without lid)
BD Falcon™ 96-well polypropylene lid fits 351190 and 353263 plates	351191
Milipore™ MultiScreen® 96-well filtration and assay plate, 1.2 µm pore	MABVN 1210 (10 pack) MABVN 1210 (50 pack)

a. Included in Consumables kit shipped with the BD FACSArray bioanalyzer

Glossary

Acquire mode	Software function of saving data.
Acquisition Control group	Contains Setup and Acquire buttons for plate acquisition, a drop-down menu to select the number of events to display, and a selection checkbox to export statistics The Acquisition Control group is located in the Plate Editor.
Acquisition Status frame	Displays Threshold Rate, Threshold Count, Processed Events, and Elapsed Time during sample acquisition
analysis	Software function of numerically and graphically manipulating data to generate statistics
APD	Avalanche photodiode Solid state optical detector in the BD FACSArray bioanalyzer that detects Far Red and NIR fluorescence
application toolbar	Displays tools used to save the current experiment, show and hide frames, open different workspaces, open new experiments, and create new experiments with the Experiment Wizard
Browser	List of all experimental data in a hierarchical view; interface for setting up Experiments and acquiring data
Coloring Control group	Contains the coloring drop-down menu where you choose how wells will be colored and the plate legend that displays the color-coding of each sample on the plate view The Coloring Control group is located in the Plate Editor.

coefficient of variation (CV)	The standard deviation of the data divided by the mean of the data; typically expressed as a percentage When applied to channel data measured on a population of cells, the CV is a measure of variation independent of the population mean.
data file	A collection of measured values from a single sample combined with text describing the sample that has been stored to disk
detector	For BD FACSArray, a device that converts light into electrical signals.
Display list	Shows the plates defined in the open experiment The Display list is located in the Plate Editor.
doublet	Two cells stuck together
dot plot	Graphical representation of two-parameter data Each axis of the plot displays values of one parameter; a dot represents an event (particle).
experiment	Group of elements used to acquire and analyze data from the bioanalyzer An experiment can include any combination of the following:, plates, samples, wells, FCS data files, keywords, plots, gates, and statistics.
flow cytometry standard (FCS)	Standard format for flow cytometer data files
frame	Software element displaying the main application components Frames can be hidden or shown, resized, and closed. The visibility, size, and position are saved when you quit the application, and are restored when you start the application the next time. You can restore the frames to the original default positions by choosing View > Reset Positions.
gate	Two-dimensional boundary defining a subset of the total sample population

histogram Graphical representation of single-parameter data

The horizontal axis of the graph represents the increasing signal intensity of the parameter, and the vertical axis represents the number of events (count).

Inspector Software interface for viewing or modifying the attributes of a single object or set of objects in the Template view or the Browser

Instrument frame Displays information about instrument status, parameter and threshold settings, laser status and power, and fluidics pressure

View settings by clicking the appropriate tab.

instrument settings Collection of values for parameters measured, parameter voltages, threshold, and the calculated parameter Log

interval One-dimensional boundary defining a subset of the total sample population

See also gate on page 362, population on page 364.

Layout for Plates Control group Provides options for adding samples to an experiment

The Layout for Plates Control group is located in the Plate Editor.

mean channel Average value of a population measured on the 0–262,144 scale.

menu bar Contains pull-down menus with commands to operate the software

optical cuvette Directs the sample stream containing cells or beads into single file where they intercept the laser beam

parameter Measurement of a cell property that is ascertained as the cell passes through the laser beam

Each parameter is the output of a single photomultiplier tube (PMT), avalanche photodiode (APD), or photodiode, measuring fluorescent or scattered light.

photodiode Solid state optical detector used in the BD FACSArray bioanalyzer to detect FSC.

Plate	Browser element representing a physical plate.
Plate Control group	Contains buttons for loading and unloading the plate The Plate Control group is located in the Plate Editor.
Plate Editor	Shows the selected plate and control frames for setting up and viewing the plate. Access the different elements by clicking the appropriate tab.
Plate view	Shows the plate view determined by the choices selected in the workspace The plate view is located in the Plate Editor.
PMT	Photomultiplier tube used in the BD FACSArray bioanalyzer to detect SSC, and Yellow and Red fluorescence
population	Data subset defined by a gate or interval
sample	Browser object representing the type of material to be analyzed, the collection date, and user-defined keywords
Select Control group	Provides a way to select wells in the plate view for acquisition The Select Control group is located in the Plate Editor.
singlet	A single cell
Setup mode	Software function of displaying events in plots, but data is not saved.
spectral overlap	Fluorescence detected in a channel other than the one for which it is intended; optical spillover.
Status box	Displays the status of the connection between the computer and the instrument, whether a 96-well plate has been loaded, the status of acquisition, and any errors that occur during acquisition The Status box is located in the Plate Editor.
stopping gate	Population for which events are to be counted
Template elements	Plots, statistics, Population Hierarchy on template view.

Template toolbar	Provides a selection arrow tool, zoom and unzoom tools, and gating tool palette.
Template view	Shows the selected analysis template you chose when you created your experiment
threshold	A trigger signal and level of discrimination to eliminate unwanted events Only events with parameter values above the threshold will be analyzed.
workspace	Views that are designed to streamline experiment preparation, setup, acquisition, and analysis

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